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INDIAN AGRICULTURAL
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JOURNAL

OF THE

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1949

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ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

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**PROCEEDINGS OF THE SIXTY-SECOND ANNUAL
MEETING OF THE ASSOCIATION OF
OFFICIAL AGRICULTURAL
CHEMISTS, 1948**

The sixty-second annual meeting of the Association of Official Agricultural Chemists was held at the Shoreham Hotel, Washington, D. C., October 11, 12, and 13, 1948.

The meeting was called to order by the President, G. H. Marsh, on the morning of October 11, at 10:00 o'clock.

**OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES
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W. F. REINDOLLAR (Bureau of Chemistry, State Department of Health, Baltimore 18, Md.), *Chairman*

SUBJECTS, REFEREES, AND ASSOCIATE REFEREES†

SUBCOMMITTEE A: H. A. HALVORSON (1950), (Department of Agriculture, Dairy, and Food, St. Paul, Minn.), *Chairman*; E. L. GRIFFIN (1952); and J. B. SMITH (1954).

FEEDING STUFFS:

Referee: L. S. WALKER, Agricultural Experiment Station, Burlington, Vt.

MINERAL MIXED FEEDS (CALCIUM AND IODINE):

A. T. Perkins, Kansas State College, Manhattan, Kans.

LACTOSE IN MIXED FEEDS:

C. W. Sievert, American Dry Milk Institute, Chicago 1, Ill

FAT IN FISH MEAL:

Maurice E. Stansby, Fish and Wildlife Service, Seattle 2, Wash.

ADULTERATION OF CONDENSED MILK PRODUCTS:

P. B. Curtis, Purdue University, Lafayette, Ind.

CRUDE FAT OR ETHER EXTRACT:

H. H. Hoffman, Department of Agriculture, Tallahassee, Fla.

MICROSCOPIC EXAMINATION:

J. A. Schrader, Agricultural Experiment Sta., Lexington 29, Ky.

FLUORINE:

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CRUDE FIBER:

W. L. HUNTER, Department of Agriculture, Sacramento 14, Calif.

PROTEIN EVALUATION IN FISH AND ANIMAL PRODUCTS:

Frank J. Kokoski, N. Y. Agricultural Experiment Station, Geneva, N. Y.

HYDROCYANIC ACID GLUCOSIDES:

E. W. Constable, State Department of Agriculture, Raleigh, N. C.

SAMPLING AND ANALYSIS OF CONDENSED BUTTERMILK:

R. E. Bergman, State Department of Agriculture, St. Paul, Minn.

TANKAGE (HIDE, HOOF, HORN, AND HAIR CONTENT):

A. T. Perkins

† Referees appointed during the year for unassigned subjects will be announced in the *Journal*.

FERTILIZERS:

Referee: F. W. Quackenbush, Agricultural Experiment Station, Lafayette, Ind.

SAMPLING AND PREPARATION OF SAMPLE:

H. R. Allen, Agricultural Experiment Station, Lexington 29, Ky.

PHOSPHORIC ACID:

K. D. Jacob, Plant Industry Station, Beltsville, Md.

MOISTURE:

W. L. Hill, Plant Industry Station, Beltsville, Md.

NITROGEN:

M. P. Etheredge, Mississippi State College, State College, Miss.

MAGNESIUM AND MANGANESE:

John B. Smith, Agricultural Experiment Station, Kingston, R. I.

ACID- AND BASE-FORMING QUALITY:

E. W. Constable

POTASH:

O. W. Ford, Purdue University, Lafayette, Ind.

SULFUR:

Gordon Hart, Department of Agriculture, Tallahassee, Fla.

COPPER AND ZINC:

H. J. Webb, A. and M. College of South Carolina, Clemson, S. C.

BORON:

G. N. Tyson, Pacific Coast Borax Co., 5 State St., Pasadena, Calif.

INERT MATERIALS:

K. G. Clarke, Division of Fertilizer and Agricultural Lime, Beltsville, Md.

ECONOMIC POISONS:

Referee: J. J. T. Graham, Production and Marketing Administration, Insecticide Division, Beltsville, Md.

TETRA ETHYL PYROPHOSPHATE:

S. A. Hall, Production and Marketing Administration, Insecticide Division, Beltsville, Md.

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DIMETHYL DITHIO CARBAMATES:

J. D. Patterson, Department of Agriculture, Salem, Oreg.

DDT:

E. E. Fleck, Bur. Entomology and Plant Quarantine, Beltsville, Md.

INSECTICIDES CONTAINING DERRIS OR CUBÉ:

F. A. Spurr, Production and Marketing Administration, Insecticide Division, Beltsville, Md.

OIL EMULSIONS:

Lloyd Keirstead, Agricultural Experiment Sta., New Haven, Conn.

DISINFECTANTS:

Referee: L. S. Stuart, Production and Marketing Adm., Insecticide Div., Beltsville, Md.

PLANTS:

Referee: E. J. Miller, Agricultural Experiment Station, East Lansing, Mich.

SAMPLING:

E. J. Miller

IODINE AND BORON:

L. K. Wood, Agricultural Experiment Station, Lexington 29, Ky.

SUGAR:

Kenneth T. Williams, Western Regional Research Laboratory, Albany, Calif.

ZINC:

E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.

COPPER AND COBALT:

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CAROTENE:

E. J. Benne

SODIUM:

Ray L. Shirley, Agricultural Experiment Sta., East Lansing, Mich.

CELLULOSE AND LIGNIN

Gordon H. Ellis, U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, N. Y.

PECTIN:

C. O. Willits, Eastern Regional Research Laboratory, Philadelphia, Pa.

STARCH:

Carroll L. Hoffpaur, Southern Regional Research Laboratory, New Orleans, La.

SPECTROGRAPHIC METHODS:

Referee: W. T. Mathis, Connecticut Agricultural Experiment Station, New Haven, Conn.

SOILS AND LIMING MATERIALS:

Referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville 16, Tenn.

HYDROGEN-ION CONCENTRATION OF SOILS:

Lannes E. Davis, Div. of Soils, Calif. Agr. Expt. Sta., Davis, Calif.

BORON AND FLUORINE

L. K. Wood

ZINC AND COPPER:

W. L. Lott, U. S. Bur. Plant Industry, Soils and Agricultural Engineering, Raleigh, N. C.

EXCHANGEABLE CALCIUM AND MAGNESIUM:

W. M. Shaw, Agricultural Experiment Station, Knoxville 16, Tenn.

EXCHANGEABLE HYDROGEN:

W. M. Shaw

EXCHANGEABLE POTASSIUM:

J. F. Reed, N. C. State College of Agriculture and Engineering, Raleigh, N. C.

PHOSPHORUS:

L. A. Dean, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Md.

STANDARD SOLUTIONS:

Referee: H. G. Underwood, Food and Drug Administration, Chicago 7, Ill.

POTASSIUM DICHROMATE SOLUTIONS:

George McClellan, Food and Drug Administration, New Orleans, La.

VITAMINS:

Referee: Chester D. Tolle, Food and Drug Administration, Washington 25, D. C.

VITAMIN A:

J. B. Wilkie, Food and Drug Administration, Washington 25, D. C.

VITAMIN A IN ANIMAL FOODS:

H. C. Schaefer, Ralston Purina Co., 835-58th St., St. Louis 2, Mo.

VITAMIN B₁:

O. L. Kline, Food and Drug Administration, Washington 25, D. C.

VITAMIN C:

W. L. Hall, Food and Drug Administration, Washington 25, D. C.

VITAMIN D—POULTRY:

Leo Friedman, Food and Drug Administration, Washington 25, D. C.

RIBOFLAVIN (FLUOROMETRIC):

H. W. Loy, Jr., Food and Drug Administration, Washington 25, D. C.

NICOTINIC ACID:

O. L. Kline, Food and Drug Administration, Washington 25, D. C.

CAROTENE:

F. W. Quackenbush

PANTOTHENIC ACID:

H. W. Loy, Jr.

FOLIC ACID:

Laura Flynn, College of Agriculture, University of Missouri, Columbia, Mo.

SUBCOMMITTEE B: G. R. CLARK (1950), (Food and Drug Administration, Washington 25, D. C.), *Chairman*; F. H. WILEY (1952), and HARRY J. FISHER (1954).

NAVAL STORES:

Referee: V. E. Grottlisch, Production and Marketing Administration, Naval Stores Division, Washington 25, D. C.

RADIOACTIVITY:

Referee: L. F. Curtiss, National Bureau of Standards, Washington 25, D. C.

QUANTUM COUNTER:

L. Costrell, National Bureau of Standards, Washington 25, D. C.

VEGETABLE DRUGS AND THEIR DERIVATIVES:

Referee: P. S. Jorgensen, Food and Drug Administration, San Francisco, Calif.

THEOBROMINE AND PHENOBARBITAL:

Daniel Banes, Food and Drug Administration, Washington 25, D. C.

AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL:

H. C. Heim, School of Pharmacy, University of Colorado, Boulder, Colo.

QUININE:

D. J. Miller, Food and Drug Administration, Buffalo 3, N. Y.

RUTIN IN TABLETS:

A. Turner, Eastern Regional Lab., U.S.D.A., Philadelphia, Pa.

ETHYLMORPHINE IN SYRUPS:

F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio

SYNTHETIC DRUGS:

Referee: F. C. Sinton, Food and Drug Administration, New York 14, N. Y.

METHYLENE BLUE:

H. O. Moraw, Food and Drug Administration, Chicago 7, Ill.

SULFANILAMIDE DERIVATIVES:

H. W. Conroy, Food and Drug Administration, Kansas City 6, Mo.

PROPADRINE HYDROCHLORIDE:

R. D. Stanley, Food and Drug Administration, Chicago 7, Ill.

CARBOMAL:

R. Hyatt, Food and Drug Administration, Cincinnati 2, Ohio

BUTACAINE SULFATE:

L. H. Welsh, Food and Drug Administration, Washington 25, D. C.

SPECTROPHOTOMETRIC METHODS:

J. Carol, Food and Drug Administration, Washington 25, D. C.

PROPYL THIOURACIL:

Gordon Smith, Food and Drug Administration, New York 14, N. Y.

PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS:

H. Rogavitz, Food and Drug Administration, New York 14, N. Y.

PYRIBENZAMINE AND BENADRYL:

H. C. Heim

SYNTHETIC ESTROGENS:

Daniel Banes, Food and Drug Administration, Washington 25, D. C.

MISCELLANEOUS DRUGS:

Referee: Iman Schurman, Food and Drug Administration, Chicago 7, Ill.

MICROSCOPIC TESTS FOR ALKALOIDS AND SYNTHETICS:

W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.

MERCURY COMPOUNDS:

M. W. Green, American Pharmaceutical Assn., Washington 25, D. C.

ORGANIC IODIDES AND SEPARATION OF HALOGENS:

V. E. Stewart, State Department of Agriculture, Tallahassee, Fla.

ALKALI METALS:

H. F. O'Keefe, Food and Drug Administration, Chicago 7, Ill.

GLYCOLS AND RELATED COMPOUNDS:

Harry Isacoff, Food and Drug Administration, New York 14, N. Y.

PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS:

C. N. Jones, Food and Drug Administration, New York 14, N. Y.

ESTRONE AND ESTRADIOL:

J. C. Molliter, Food and Drug Administration, Washington 25, D. C.

METHYL ALCOHOL:

J. F. Guymon, Agricultural Experiment Sta., College of Agriculture, Davis, Calif.

COSMETICS:

Referee: G. R. Clark, Food and Drug Administration, Washington 25, D. C.

COSMETIC CREAMS:

C. F. Bruening, Food and Drug Administration, Baltimore 2, Md.

COSMETIC POWDERS:

Helen C. Barry, Food and Drug Administration, New Orleans 16, La.

COSMETIC SKIN LOTIONS:

H. R. Bond, Food and Drug Administration, Kansas City 6, Mo.

DEODORANTS AND ANTI-PERSPIRANTS:

S. H. Newburger, Food and Drug Administration, Baltimore 2, Md.

DEPILATORIES:

S. H. Newburger

HAIR DYES AND RINSES:

S. W. Newburger

MASCARA, EYEBROW PENCILS, AND EYE SHADOW:

Paul W. Jewel, Max Factor and Company, Hollywood, Calif.

MOISTURE IN COSMETICS:

J. F. Weeks, Food and Drug Administration, New Orleans 16, La.

SUN TAN PREPARATIONS:

E. Hoshall, Food and Drug Administration, Baltimore 2, Md.

COAL-TAR COLORS:

Referee: K. A. Freeman, Food and Drug Administration, Washington 25, D. C.
ACETATES, CARBONATES, HALIDES, AND SULFATES IN CERTIFIED COAL-TAR COLORS:

A. T. Schram, National Aniline Division, P.O. Box 975, Buffalo 5, N. Y.

BUFFERS AND SOLVENTS IN TITANIUM TRICHLORIDE TITRATIONS:

S. S. Forrest, Food and Drug Administration, Washington 25, D. C.

ETHER EXTRACT IN COAL-TAR COLORS:

S. S. Forrest

HALOGENS IN HALOGENATED FLUORESCENS:

J. H. Jones, Food and Drug Administration, Washington 25, D. C.

IDENTIFICATION OF CERTIFIED COAL-TAR COLORS:

Rachel Sclar, Food and Drug Administration, Washington 25, D. C.

VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS:

Alice B. Caemmerer, Food and Drug Administration, Washington 25, D. C.

NON-VOLATILE UNSULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:

L. S. Harrow, Food and Drug Administration, Washington 25, D. C.

SULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:

N. Ettlestein, Food and Drug Administration, Washington 25, D. C.

UNSULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:

H. Holtzman, Ansbacher-Siegle Corp., Rose Bank, Staten Island, N. Y.

SULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:

W. C. Bainbridge, H. Kohnstamm Company, Brooklyn 31, N. Y.

INTERMEDIATES DERIVED FROM PHTHALIC ACID:

C. Graichen, Food and Drug Administration, Washington 25, D. C.

MIXTURES OF COAL-TAR COLORS FOR DRUG AND COSMETIC USE:

W. C. Bainbridge

LAKES AND PIGMENTS:

C. Graichen

SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS:

J. H. Jones

SUBSIDIARY DYES IN D&C COLORS:

L. Koch, H. Kohnstamm and Company, Brooklyn 31, N. Y.

HEAVY METALS IN COAL-TAR COLORS:

C. Stein, Food and Drug Administration, Washington 25, D. C.

ARSENIC IN COAL-TAR COLORS:

L. S. Harrow

SUBSIDIARY DYES IN FD&C COLORS:

M. Dolinsky, Food and Drug Administration, Washington 25, D. C.

HYGROSCOPIC PROPERTIES OF COAL-TAR COLORS:

C. Stein

BOILING RANGE OF PSEUDO-CUMIDINE XYLIDINE IN CERTIFIED COAL-TAR COLORS:

L. S. Harrow

SUBCOMMITTEE C: J. O. CLARKE (1950), (Food and Drug Administration, Washington 25, D. C.), *Chairman*; P. A. CLIFFORD (1952), and A. H. ROBERTSON (1954).

PROCESSED VEGETABLE PRODUCTS:

Referee: V. B. Bonney, Food and Drug Administration, Washington 25, D. C.

QUALITY FACTORS:

R. D. Lovejoy, Food and Drug Administration, Washington 25, D. C.

MOISTURE IN DRIED VEGETABLES:

B. Makover, Western Regional Research Laboratory, U. S. Department of Agriculture, Albany 6, Calif.

CATALASE IN FROZEN VEGETABLES:

B. M. Gutterman, Food and Drug Administration, Washington 25, D. C.

PEROXIDASE IN FROZEN VEGETABLES:

M. A. Joslyn, College of Agr., Univ. of Calif., Berkeley 4, Calif.

FILL OF CONTAINER METHODS (FOODS, DRUGS, AND COSMETICS):

Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.

COFFEE AND TEA

Referee:

COLORING MATTERS IN FOODS

Referee: C. F. Jablonski, Food and Drug Administration, New York 14, N. Y.

DAIRY PRODUCTS:

Referee: W. Horwitz, Food and Drug Administration, Minneapolis 1, Minn.

PHOSPHATASE TEST IN DAIRY PRODUCTS:

W. Horwitz

ASH IN MILK AND EVAPORATED MILK:

Guy G. Frary, State Chemical Laboratory, Vermillion, S. Dak.

SAMPLING, FAT, AND MOISTURE IN HARD CHEESES:

W. Horwitz

ACIDITY OF MILK:

Guy G. Frary

PREPARATION OF BUTTER SAMPLES:

A. L. Weber, Food and Drug Administration, New York 14, N. Y.

TESTS FOR RECONSTITUTED MILK:

SERUM TESTS:

Henry J. Hoffman, Minnesota Dept. of Agriculture, St. Paul, Minn.

FAT IN DAIRY PRODUCTS:

Ernest O. Herreid, Illinois Agricultural Expt. Sta., Urbana, Ill.

SAMPLING, AND PREPARATION OF SAMPLE, OF SOFT CHEESES:

Sam Perlmutter, Food and Drug Administration, Minneapolis, Minn.

FROZEN DESSERTS:

H. M. Boggs, Food and Drug Administration, Philadelphia, Pa.

EGGS AND EGG PRODUCTS:

Referee: F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio

ADDED GLYCEROL:

George E. Keppel, Food and Drug Administration, Minneapolis 1, Minn.

ACIDITY OF FAT:

H. Van Dame, Food and Drug Administration, Cincinnati 2, Ohio

FAT:

L. C. Mitchell, Food and Drug Administration, Minneapolis, Minn.

AMMONIA NITROGEN:

E. B. Boyce, State Dept. Public Health, Boston 33, Mass.

EXTRANEEOUS MATERIALS IN FOODS AND DRUGS:

Referee: K. L. Harris, Food and Drug Administration, Washington 25, D. C.

DRUGS, SPICES, AND MISCELLANEOUS MATERIALS:

W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.

DAIRY AND EGG PRODUCTS:

K. L. Harris

NUT PRODUCTS:

Maryvee G. Yakowitz, Food and Drug Administration, Washington 25, D. C.

BAKED PRODUCTS, CEREALS, AND CONFECTIONERY:

J. F. Nicholson, Food and Drug Administration, Washington 25, D. C.

BEVERAGE MATERIALS:

F. A. Hodges, Food and Drug Administration, Washington 25, D. C.

FRUIT PRODUCTS:

W. G. Helsel, Food and Drug Administration, Washington 25, D. C.

VEGETABLE PRODUCTS:

F. R. Smith, Food and Drug Administration, Washington 25, D. C.

SEDIMENT TESTS (MILK AND CREAM):

C. R. Joiner, Food and Drug Administration, St. Louis, Mo.

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL INDICES):

Referee: W. I. Patterson, Food and Drug Administration, Washington 25, D. C.

FISH PRODUCTS:

Fred Hillig, Food and Drug Administration, Washington 25, D. C.

DAIRY PRODUCTS:

Fred Hillig

SHELLFISH:

R. E. Duggan, Food and Drug Administration, New Orleans 16, La.

APPLE PRODUCTS:**EGG PRODUCTS:**

Fred Hillig

BLACKHEART IN PINEAPPLE:

W. O. Winkler, Food and Drug Administration, Washington 25, D. C.

GELATINE, DESSERT PREPARATIONS, AND MIXES:

Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.

JELLY STRENGTH:

Paul A. Kind, Kind-Knox Gelatine Co., Camden, N. J.

GELATINE AND GELATINE DESSERTS (COMPOSITION):

Joseph H. Cohen, General Foods Corporation, Woburn, Mass.

FISH AND OTHER MARINE PRODUCTS:

Referee: A. M. Allison, Food and Drug Administration, Boston 10, Mass.

TOTAL SOLIDS AND ETHER EXTRACT:

Menno D. Voth, Food and Drug Administration, Boston 10, Mass.

GUMS IN FOODS:

Referee: F. Leslie Hart, Food and Drug Administration, Los Angeles 15, Calif.
CHEESE:

M. J. Gnagy, Food and Drug Administration, Los Angeles, Calif.

FROZEN DESSERTS:

F. Leslie Hart

CACAO PRODUCTS:

Flora G. Mendelsohn, Food and Drug Administration, Los Angeles 15, Calif.

CATSUP AND RELATED TOMATO PRODUCTS:

E. W. Coulter, Food and Drug Administration, Chicago 7, Ill.

MEAT AND MEAT PRODUCTS:

Referee: Roger M. Mehurin, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.

SOYBEAN FLOUR IN MEAT PRODUCTS:

O. L. Bennett, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.

DEFATTED MILK SOLIDS IN MEAT PRODUCTS:

CREATIN IN MEAT PRODUCTS:

J. M. McCoy, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.

HORSEMEAT IN GROUND MEAT:

C. E. Hynds, State Food Laboratory, Albany, N. Y.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS:

Referee: H. J. Wichmann, Food and Drug Administration, Washington 25, D. C..

CADMIUM:

A. K. Klein, Food and Drug Administration, Washington 25, D. C.

COPPER:

W. C. Stammer, Continental Can Company, Inc., Chicago 39, Ill.

ZINC:

O. R. Alexander, American Can Company, Maywood, Ill.

MERCURY:

A. K. Klein

DDT AS SPRAY RESIDUE ON FOODS:

R. H. Carter, Bureau of Entomology and Plant Quarantine, Beltsville, Md.

INSECTICIDES IN CANNED FOODS:

W. A. Britton, Beechnut Packing Co., Canajoharie, N. Y.

PARATHION:

P. A. Clifford, Food and Drug Administration, Washington 25, D. C.

MICROBIOLOGICAL METHODS:

Referee: G. G. Slocum, Food and Drug Administration, Washington 25, D. C.

CANNED MEATS:

M. L. Laing, Armour & Company, Chicago 9, Ill.

CANNED ACID FOODS:

A. P. Dunningan, Food and Drug Administration, Washington 25, D. C.

CANNED VEGETABLES:

C. W. Bohrer, Natl. Cannery Assn., Washington, D. C.

EGGS AND EGG PRODUCTS:

M. T. Bartram, Food and Drug Administration, Washington 25, D. C.

NUTS AND NUT PRODUCTS:

William R. North, Food and Drug Administration, Washington 25, D. C.

FROZEN FRUITS AND VEGETABLES:

H. E. Goresline, Production and Marketing Administration, Poultry Division, Washington 25, D. C.

SUGAR:

E. J. Cameron, Natl. Canners Assn., Washington, D. C.

CANNED FISHERY PRODUCTS:

L. R. Shelton, Jr., Food and Drug Administration, Washington 25, D. C.

MICROCHEMICAL METHODS:

Referee: C. O. Willits, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa.

ELEMENTAL ANALYSIS:

C. L. Ogg, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa.

NUTS AND NUT PRODUCTS:

Referee: A. M. Henry, Food and Drug Administration, Atlanta 3, Ga.

OILS, FATS, AND WAXES:

Referee: J. Fitelson, Food and Drug Administration, New York 14, N. Y.

UNSAAPONIFIABLE MATTER:

Gardner Kirsten, Food and Drug Administration, New York 14, N. Y.

PEANUT OIL:

Gardner Kirsten

ANTIOXIDANTS:

S. Kahan, Food and Drug Administration, New York 14, N. Y.

PRESERVATIVES AND ARTIFICIAL SWEETENERS:

Referee: Margarethe Oakley, State Department of Health, Baltimore 18, Md.

QUATERNARY AMMONIUM COMPOUNDS:

John B. Wilson, Food and Drug Administration, Washington 25, D. C.

MONOCHLORACETIC ACID:

JOHN B. WILSON

FORMALDEHYDE:

Howard Bennett, Food and Drug Administration, New Orleans, La.

MOLD-INHIBITORS, PROPIONATES:

L. H. McRoberts, Food and Drug Administration, San Francisco 2, Calif.

THIOUREA:

W. O. Winkler, Food and Drug Administration, Washington 25, D. C.

ARTIFICIAL SWEETENERS:

William S. Cox, Food and Drug Administration, Atlanta, Ga.

SPICES AND OTHER CONDIMENTS:

Referee: S. Alfend, Food and Drug Administration, St. Louis 1, Mo.

VINEGAR:

J. H. C. Loughrey, Food and Drug Administration, Boston, Mass.

VOLATILE OIL IN SPICES:

N. A. Carson, Food and Drug Administration, St. Louis, Mo.

SUGAR, ASH, AND PUNGENT PRINCIPLES IN MUSTARDS:

F. M. Garfield, Food and Drug Administration, St. Louis 1, Mo.

PREPARATION OF SAMPLE, AND FAT IN MAYONNAISE AND SALAD DRESSING:

Juanita E. Breit, Food and Drug Administration, Cincinnati 2, Ohio

SUBCOMMITTEE D: KENNETH L. MILSTEAD (1950) (Food and Drug Administration, Cincinnati, Ohio), *Chairman*; J. Walter Sale (1952); and C. S. Ferguson (1954)

ALCOHOLIC BEVERAGES

Referee: J. Walter Sale, Food and Drug Administration, Washington 25, D. C.
YEAST (TOTAL AND YEAST SOLIDS):

Robert I. Tenney, Wahl-Henius Inst., 64 E. Lake St., Chicago, Ill.

HOPS:

D. E. Bullis, Oregon State College, Corvallis, Oreg.

INORGANIC ELEMENTS IN BEER:

W. C. Stammer, Continental Can Company, Inc., Chicago, Ill.

COLOR AND TURBIDITY IN BEER:

B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Mo.

DISTILLED SPIRITS (OBSCURATION METHOD FOR PROOF AND TEST FOR AGING):

G. F. Beyer, Bureau of Internal Revenue, Washington 25, D. C.

CHROMATOGRAPHIC ABSORPTION OF WINES:

Peter Valaer, Bur. Internal Revenue, Washington 25, D. C.

CARAMEL IN ALCOHOLIC BEVERAGES:

Peter Valaer

CORDIALS AND LIQUEURS:

John B. Wilson, Food and Drug Administration, Washington 25, D. C.

FUSEL OIL IN DISTILLED SPIRITS (OFFICIAL METHOD):

G. F. Beyer

CARBON DIOXIDE IN BEER:

Irwin Stone, Wallerstein Laboratories, New York 16, N. Y.

METHANOL:

J. F. Guymon, Agr. Expt. Station, College of Agriculture, Davis, Calif.

CACAO PRODUCTS:

Referee: W. O. Winkler, Food and Drug Administration, Washington 25, D. C.

LECITHIN:

J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.

MALT SOLIDS:

E. W. Meyers, Hershey Chocolate Company, Hershey, Pa.

PECTIC ACID:

W. O. Winkler

CACAO INGREDIENTS:

W. O. Winkler

LACTOSE:

Donald G. Mitchell, Walter Baker Co., Dorchester 24, Mass.

FAT:

Carl Stone, Food and Drug Administration, Cincinnati, Ohio

CEREAL FOODS:

Referee: V. E. Munsey, Food and Drug Administration, Washington 25, D. C.

STARCH IN RAW AND COOKED CEREALS:

FAT ACIDITY IN GRAIN, FLOUR, CORN MEAL, AND WHOLE WHEAT FLOUR

Lawrence Zeleny, Agricultural Research Center, Beltsville, Md.

BENZOIC ACID IN FLOUR:

V. E. Munsey

MILK SOLIDS AND BUTTERFAT IN BREAD:

V. E. Munsey

PROTEOLYTIC ACTIVITY OF FLOUR:

Byron S. Miller, Federal Hard Wheat Quality Laboratory, Manhattan, Kans.

SOYBEAN FLOUR:

W. L. Taylor, General Mills, Inc., Minneapolis, Minn.

PHOSPHATED FLOUR:

Frank H. Collins, Food and Drug Administration, Cincinnati 2, Ohio

BAKED PRODUCTS (MOISTURE, ASH, PROTEIN, FAT, AND CRUDE FIBER):

R. P. Smith, National Biscuit Co., 449 W. 14th St., New York, N. Y.

MOISTURE IN SELF-RISING FLOUR AND IN PANCAKE, WAFFLE, AND DOUGH-NUT MIXES:

S. Williams, Food and Drug Administration, Cincinnati, Ohio

BROMATES IN FLOUR:

W. L. Rainey, Commander-Larabee Milling Co., Minneapolis, Minn.

UNSAAPONIFIABLE MATTER AND STEROLS IN NOODLES AND BAKERY PRODUCTS:

V. E. Munsey

ALBUMEN IN NOODLES AND MACARONI PRODUCTS:

V. E. Munsey

BAKING POWDERS AND BAKING CHEMICALS:

Referee: V. E. Munsey, Food and Drug Administration, Washington 25, D. C.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:

Referee: John B. Wilson, Food and Drug Administration, Washington 25, D. C.

BETA-IONONE:

John B. Wilson

LEMON OILS AND EXTRACTS:

John B. Wilson

ORGANIC SOLVENTS IN FLAVORS:

R. D. Stanley, Food and Drug Administration, Chicago 7, Ill.

EMULSION FLAVORS:

John B. Wilson

MAPLE FLAVOR CONCENTRATES AND IMITATIONS:

J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.

DIACETYL:

John B. Wilson

VANILLA EXTRACTS AND IMITATIONS:**FRUITS AND FRUIT PRODUCTS:**

Referee: R. A. Osborn, Food and Drug Administration, Washington 25, D. C.

TITRATION OF ACIDS:

H. M. Bollinger, Food and Drug Administration, Los Angeles 15, Calif.

FRUIT ACIDS:

L. W. Ferris, Food and Drug Administration, Buffalo, N. Y.

FRUIT AND SUGAR IN FROZEN FRUIT:

C. G. Hatmaker, Food and Drug Administration, Washington 25, D. C.

WATER-INSOLUBLE SOLIDS:

R. A. Osborn

SUGARS AND SUGAR PRODUCTS:

Referee: C. F. Snyder, National Bureau of Standards, Washington 25, D. C.

DRYING METHODS:

Lester D. Hammond, National Bureau of Standards, Washington 25, D. C.

DENSIMETRIC AND REFRACTOMETRIC METHODS:

C. F. Snyder

HONEY (FREE ACID AND COMMERCIAL SYRUP ADULTERANTS):

George P. Walton, Eastern Regional Research Laboratory, U. S. Dept.
Agriculture, Philadelphia, Pa.

CONFECTIONERY:

C. A. Wood, Food and Drug Administration, New York 14, N. Y.

REDUCING SUGARS:

Emma J. McDonald, National Bureau of Standards, Washington 25, D. C.

CORN SIRUP AND CORN SUGAR:

G. T. Peckham, Jr., Clinton Company, Clinton, Iowa

COLOR AND TURBIDITY IN SUGAR PRODUCTS:

J. F. Brewster, National Bureau of Standards, Washington 25, D. C.

MICRO-SUGAR METHODS:

Betty L. Kostakos, National Bureau of Standards, Washington 25, D. C.

STARCH CONVERSION PRODUCTS:

WATERS, BRINE, AND SALT:

Referee:

BORON IN WATER:

FLUORINE IN SALT:

MEMBERS AND VISITORS PRESENT, 1948 MEETING

- Adams, J. Richard, Spencer Chemical Co., Baltimore St., Kansas City 2, Mo.
Alexander, Lyle T., Bur. Plant Industry, Soils, & Agr. Engineering, Beltsville, Md.
Alexander, O. R., American Can Co., 11th Ave. & St. Charles Road, Maywood, Ill.
Alfend, Chief Chemist, Food & Drug Adm., St. Louis Station, St. Louis 1, Mo.
Allen, H. R., Ky. Agricultural Expt. Station, Lexington 29, Ky.
Allen, Raymond N., Dir. Biological Dept., Gorton Pew Fisheries Co., Ltd., Gloucester, Mass.
Allison, Andrew M., Chief Chemist, Boston Station, Food & Drug Adm., Boston, Mass.
Amick, C. Harold, Dir. Food & Drug Div., State Dept. of Agriculture, Charleston, W. Va.
Anderson, Myron S., Bur. Plant Industry, Soils, & Agr. Engineering, Beltsville, Md.
Anwar, M. H., Research Dir., Coca-Cola Expt. Corp., 515 Madison Ave., New York, N. Y.
Austin, W. R., Armour Test Works, Nashville 2, Tenn.
Axley, John, University of Maryland, College Park, Md.
- Bacher, A. A., 735 N. Water St., Milwaukee, Wis.
Bacon, C. W., Plant Industry, Soils, & Agr. Engineering, Beltsville, Md.
Bailey, Lorin H., 3904 McKinley St., Washington 15, D. C.
Baker, Warren S., Dir. Research, Chas. M. Cox Co., 177 Milk St., Boston 9, Mass.
Banes, Daniel, Food & Drug Administration, Washington 25, D. C.
Barnhart, G. M., Chief Chemist, Mo. Dept. of Agriculture, Jefferson City, Mo.
Bartlett, L. E., Park & Pollard Co., 356 Hertel Ave., Buffalo 7, N. Y.
Bates, D. B., Smith-Douglas Co., Board of Trade Building, Norfolk 1, Va.
Batton, H. C., Swift & Co., Plant Food Div., Norfolk, Va.
Beacham, C. M., Food & Drug Administration, Washington 25, D. C.
Benne, Erwin J., Agricultural Expt. Station, State College, East Lansing, Mich.
Berry, Rodney C., State Chemist, Va. Dept. of Agr., State Office Bldg., Richmond 19, Va.
Beyer, G. F., Bureau of Internal Revenue, Washington 1, D. C.
Bidez, P. R., State Chemical Laboratory, 325 Magnolia St., Auburn, Ala.
Black, A., E. R. Squibb & Sons, New Brunswick, N. J.
Blackwell, A. T., Davison Chemical Corp., Baltimore, Md.
Blanchard, J. F., Supt. Lab. Food & Drugs, National Health & Welfare, Winnipeg, Canada
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PRESIDENT'S ADDRESS*

COMPOSITION FOUND BY ANALYSIS WITH THERAPEUTICAL CLAIMS FOR SOME PROPRIETARY MEDICINES

By **GEORGE H. MARSH** (Director Chemistry Division, Alabama Department of Agriculture and Industries, Montgomery 2, Ala.)

The compounding of medicine is an old art. The people of ancient times had a meager knowledge of the medicinal properties of herbs and roots and their therapeutical action upon the human body. They gathered certain herbs and roots and made decoctions and infusions from some of them, chewed some, smoked some, and took some of them in powdered form. At the same time there existed somewhat of a halo of mystery about the action of medicine on the human body. That same idea which originated with the art of compounding of medicine still prevails in the minds of many people. They believe that there is some mysterious something in certain medicines. They cannot explain it to you, but still it exists in their minds.

This halo of mystery, or conception of medicinal values, has been a retarding factor through the years in getting proper medicinal aid to many people and it continues to be a hindrance even today. It is the controlling reason why many people do not heed the advice for correct and proper medication, but rather follow the mystic view they have of medicines and self medication, to the detriment of their health and even sometimes to the loss of life itself. This idea of the mystery of medicines and their healing power on the human body makes it profitable for those who are inclined to make use of the traffic in medicines for profit and, unfortunately, many are willing to take advantage of their fellow man's weakness: all for the sake of personal material gain. This is so well illustrated by the type of advertising, and the extensive therapeutical claims made so often.

The analysis for composition of many of the medicines which have been found on sale is a very broad and difficult field, of course, for the Drug Chemist in control work whose task it is to ascertain the composition of many of them. This is especially true in the case of medicines made up by the itinerant drug or medicine man, as they are called. It is likewise true in the case of the medicines put up in many cases by the small manufacturer, who puts up a medicine and jobs it out through small grocery stores, etc.

The seriousness of the situation is that in so many of the cases the party making the medicine is one who knows nothing about drugs, chemistry, physiology, or therapeutics. Even if he is honest in purpose, still he

* Presented before the Annual Meeting of the Association of Official Agricultural Chemists, held at the Shoreham Hotel, Washington, D. C., October 11, 12, 13, 1948.

knows nothing about what value the medicine may or may not have. The person's mind purchasing the medicine is often filled with the idea of mystery, and he believes in some way it will alleviate his pain and suffering and per chance relieve him of his ailment. It is surely a case of the blind leading the blind as they grope around in darkness.

It is no easy task to cope with this situation in drug control, which we have briefly described. If there was the requirement that certain information be filed with the Drug Control Official, such as the composition of the medicine, copy of the label, and a copy of the proposed advertising matter, *before* the medicine is offered for sale, it would be very valuable in the control in the traffic of these medicines and in rendering of far greater protection of the health of the public. There are many of such medicines being sold. The business of concocting of them seems to be a never ending business; for as soon as one has been apprehended and put out of business, others seem to spring up in his place. It seems that those who know the least about medicine, pharmacy, chemistry, physiology, or therapeutics are the ones who manufacture and sell such medicines.

I shall refer to some specific cases where claims were made or implied as to the value of a remedy for certain diseases, and then give the composition of the medicine as was found by analysis in the laboratory.

(1) "TU-BU-KU"

This medicine was a colorless liquid sold in 6 oz. bottles, bearing directions on the label to take as a relief for all coughs. The name might imply that the product is to be used as a remedy for tuberculosis, since in most lung or throat infections of this disease a cough usually accompanies the disease. A sample consisting of two 6 oz. bottles was collected and analyzed in the laboratory. The Drug Chemist had no knowledge of the composition of the medicine since no ingredients were listed on the label, and further he had no knowledge of a medicine or any medicinal ingredient that was a remedy for tuberculosis. He began his search for the composition of the medicine, and his first clue was the odor of peppermint. A test showed that the medicine contained menthol in a very small amount. In carrying out the test for menthol, an indication was noticed of the presence of alcohol in the medicine. Tests were made and the presence of alcohol confirmed. A quantitative determination was made which showed that the medicine contained 10 per cent by volume of alcohol. In the test for alcohol an indication was noticed that the medicine contained a trihydroxy alcohol, and confirmatory tests showed this alcohol to be glycerine. A quantitative determination proved the presence of 3 per cent by volume of glycerine. The only other ingredient found present was water: 87 per cent by volume. The medicine actually consisted of an aqueous solution containing 10 per cent by volume of ethyl alcohol and 3 per cent by volume of glycerine, flavored with menthol. Fortunately, soon after

the analysis was completed and reported, the product was withdrawn from the market and so far as I know never came back on the market in our section. It was not possible to correlate any relation between the therapeutical implication in the name of the product on the label, and the composition found in the laboratory analysis of the product.

(2) "PELLAGRA REMEDY"

A sample of 4 oz. of this medicine was collected. It was a grayish powder intermingled throughout with a coarser black powder. The Drug Chemist, not having a knowledge of any medicine that was a specific remedy for pellegra, it was difficult for him to decide just what to test for in the medicine. It was decided to test first to ascertain what the coarser black powder scattered throughout the samples was. A portion was taken and sieved, and it was found not difficult to separate a portion of the black particles out from the grayish powder. Some of the separated coarser black particles were placed in a crucible, and heat applied. These particles proved to be charcoal not very finely pulverized. A qualitative test was carried out on a portion of the sample for heavy metals, and it was soon found that the medicine contained iron. A quantitative test was run to find the total iron content. It was then decided that, since the powder was water soluble, it was a salt; so tests were made to ascertain what salt or salts were present. It was found that it was a sulphate, and that the iron was in a ferrous form with only a trace of ferric iron present. No other salt was found to be present in the medicine. The composition of the product was calculated from the percentage of ferrous iron and the sulphate radical, and the product was found to be practically pure exsiccated ferrous sulphate, containing less than $\frac{1}{2}$ of 1 per cent of charcoal in the original medicine. It was concluded that the exsiccated ferrous sulphate was mixed with the small amount of charcoal to mask the appearance of the pure salt and carry that halo of mystery, in order to sell the medicine for extensive profits. The 4 oz. package sold for \$1.00 a box. Pellagra was a rather common disease at the time among the people where this product was being sold. It was natural that the people were grabbing at something they hoped would be a relief for their ailment. There was little knowledge at the time of a scientific remedy for the treatment of the disease, pellagra, since it had not yet been found that it was a deficiency disease. The remedy was sold by an itinerant medicine man who was doing quite a profitable business until his product was seized; and so far as I know he discontinued the medicine business.

(3) "CANCER REMEDY"

The medicine was labeled "Rock of All Ages." A sample was collected consisting of two 4 oz. boxes. The article was found to be a dark granular powder, almost black. The label did not show the composition, as is so

generally the case with most of such medicines. It was extensively labeled as to what the medicine would do. It also bore labeling as to how to prepare the medicine for use. The powder itself was not to be taken, but the contents of the 4 oz. box were to be macerated in a quart of water for several hours, or overnight, and then the liquid poured off into another quart bottle, stoppered, and kept for administering as a remedy for cancer and almost any other disease one might become afflicted with. The directions for dosage, but no time limit as to when to stop taking the medicine when once begun, were mentioned on the label. An analysis of the solution, obtained according to directions of preparation, showed but one ingredient in any appreciable quantity, and that was ferric sulphate. There was a trace of another substance, namely arsenic, but not in sufficient quantities to be of material consequence. An analysis of the original solid substance, or so-called "Mineral Rock of All Ages," showed that it contained sand and clay and a small amount of ferrous sulphate and ferric sulphate. The seller of this product was a local itinerant medicine man; however, he jobbed some of this medicine to grocery stores and occasionally to a country drug store. He was convicted three different times for selling the medicine. He was put in jail overnight, and fined \$50.00 and court costs, the first time. For the second offence he was convicted and fined \$100.00 and court costs, and given a 6-month's prison sentence, which was suspended. The third time he was convicted and fined \$100.00 and court costs, and given a 6-month's prison sentence. The case was appealed to the Supreme Court, where the lower court was upheld. The medicine man died about the time the Supreme Court's decision was handed down. Each trial was in a separate county.

(4) "LINIMENT ANTISEPTIC"

This medicine was put up in 3 oz. bottles and bore the following statement on its label: "Liniment Antiseptic will relieve rheumatic pains, blood poison, toothache, does relieve pyorrhea, good for tonsillitis, asthma, relieves backache, sprains, soreness of body, hard of hearing, boils, bunions, corns, sore feet, good for colitis, fine for weak eyes, for colds, catarrh of the head, headache and flu. It kills all germs, etc." Two bottles were collected for a sample. The Drug Chemist, having no knowledge of a specific remedy for relief of all rheumatic pains, pyorrhea, flu, asthma, colitic, or all the other diseases enumerated on the label of this medicine, found it all the more difficult to know where to begin analysis or what to look for in the medicine. The first indication that was observed was the odor of camphor contained in the product: the article being a solution, also indicated that it contained alcohol. Tests were made which proved the presence of both camphor and alcohol in the medicine. The color of the liquid was amber, so that indicated the presence of still other substances. A quantitative test was made for the percentage of alcohol,

and also for the percentage of camphor; and qualitative tests were made for iodine which proved the presence of iodine. There was about 84 per cent by volume of alcohol present in the article and, from the amount of iodine present, the indication was that about 3 per cent of tincture of iodine was added to spirits of camphor to make up the remedy. It was not possible to correlate the therapeutical claims of the label with the composition found by analysis of the medicine. The label of this medicine has been corrected to such an extent that one would hardly recognize the product except by its color and the name of the manufacturer.

(5) "HIGH POWER MAJIC INHALER"

This was a medicine put up in $\frac{1}{2}$ oz. bottles. Two bottles were collected for samples. The following statement appeared on the label: "High Power Majic Inhaler. Try Majic for headaches, head colds, sinus troubles, hay fever, asthma. Directions—Just inhale the fumes from the cork, for headaches rub the cork over the forehead. Keep corked when not in use." The analysis of the contents of this medicine showed it to consist of a mixture of powdered leaves, cinnamon bark, calamus root, hulls of anise, caraway, coriander seed and bay leaves, saturated with volatile oil of mustard. It was a powerful remedy. It required only a small portion to be very irritating. It was a dangerous medicine being sold by an itinerant medicine man. He was apprehended but received only a moderate fine; however he discontinued business in the section where the sample was collected.

(6) "LONESOME PINE HEALING OIL"

The following statement appeared on the label: "In case you have kidney trouble or brights disease write in for treatment with Lonesome Pine Healing Oil." Another statement on the label was as follows: "For cuts, colds, ulcers, burns, bruises, toothaches will relieve pyorrhea, also for flu, asthma, hay fever, poison oak, ring worms, athletes foot, etc." Three 2 oz. bottles were collected as a sample. Examination of these bottles showed them to contain a straw-colored oil. Further analysis showed it to be an impure rosin oil, or a partially resinified pine oil. No other substances were found to be present in the product. It was not possible to correlate the claims on the label for the therapeutical value of this product, with the composition found by analysis in the laboratory. The analysis of this product was not as difficult as many others, since it was indicated in the name of the product that it contained some form of pine oil, and that proved to be true.

(7) "A MALARIA REMEDY"

This was a liquid medicine, colored. Claims were made that the article contained quinine sulphate as active ingredient, with other ingredients

having tonic properties. The claim on the label was that the dosage recommended, which was a tablespoonful, contained 5 grains of quinine sulphate. The article was sampled and examined in the laboratory. Quinine sulphate was found to be present. No other active ingredient, except a small amount of gentian, was found in the medicine. A quantitative determination revealed the presence of only 2.50 grains of quinine sulphate per tablespoonful of the medicine, instead of the 5 grains claimed to be present. It is rather generally accepted that quinine sulphate is a specific for Malaria Plasmosis if given in certain recognized dosage. But a person who knew that fact and knew the correct dosage, and who depended upon expected results after taking this remedy, would have been sadly disappointed. The article contained the declared active ingredient, but not in the amount claimed. A case of misbranding, also a subtle type of declaration of the dosage, made the article deceptive, since 5 grains is the common accepted amount given each time to adults until an ample amount has been administered to give the desired effect in allaying malaria.

(8) "U. S. TONIC"

This was a medicine put up in 12-ounce bottles, labeled as "U. S. Tonic" and bearing a small flag on the bottle. The flag was a paper design of the stars and stripes. The name "Washington D. C." was also on the label. The dosage to be given was about the only other information on the label. It was not possible to collect an unopened bottle of this medicine, but two bottles which had been opened were collected from two different persons who had purchased a bottle at the price of \$5.00. The examination revealed that the article was principally water with a little gentian and fluid extract of cascara sagrada. The reason, probably, for leaving the claims for use of the medicine off of the label was because that information was given orally, according to the purchasers' statements, by the itinerant medicine man. He traveled in a rather nice Buick Coupe Automobile, dressed quite nicely and wore a white coat similar to that worn by doctors in their offices. He also carried a hand bag very similar to those commonly used by doctors for carrying certain equipment and supplies in their professional calls. He carried a stethoscope in this bag; also a few bottles of the U. S. Tonic when he approached the patient in his home. His first approach was that he was a Government agent sent out to make check-ups on the people as to their state of health. He quickly told the prospective patient that there was no charge for the examination. With this approach, it was not difficult to obtain permission to proceed with the check-up on the patient. The agent opened up the hand bag, took out the stethoscope and began the examination of the patient as to state of health and general condition. When the check-up was completed he informed the patient what he had found wrong. He then reiterated his statement that there was no charge for the examination, but that the patient should have some

medicine. He then took out a bottle of the U. S. Tonic with the U. S. Flag on the bottle and announced that was the remedy, or medicine, needed by the patient in order to correct his or her trouble. The patient was informed that the price was \$5.00 per bottle; also that there was only a limited amount of it available for sale. The examination in the laboratory by the Drug Chemist showed the article to contain a very small amount of active ingredients. The trouble was, the procedure used made it difficult for the type of people who were approached to refuse to purchase the medicine. This agent traveled rapidly, and we found only two places in the state where he operated. His visits at these two sections were in rural communities during harvest time. It was also during the last war and we have not been able to find where he has been in the state since.

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will follow in their usual order.

Third Day

WEDNESDAY—AFTERNOON SESSION

REPORT OF COMMITTEE ON CLASSIFICATION OF METHODS

Your committee has received much helpful comment on the feasibility of the six recommendations made at the 1947 meeting.* It appears that many tentative methods are well on their way toward either adoption as official or deletion, depending on their worth. Accordingly, one proposed amendment to the by-laws abolishes the "tentative" classification. This covers recommendations (1) and (2).

Recommendation (3) provided for a "pending official" classification. This, in the light of the past year's experience, seems unnecessary, and indeed undesirable. It is convenient to discuss (3) in connection with (4), which dealt with methods that have long been in the "official, first action" classification. An amendment to the by-laws is proposed providing adoption of methods as "first action" and "official." Those tentative methods not deleted or not adopted as official at the 1949 meeting could be adopted as "first action" at that time on recommendation of the appropriate referee, provided they had received collaborative study. All "official, first action" methods remaining in that status at the close of the 1949 meeting would automatically become "first action" methods.

Recommendation (5) dealt with methods of a screening or sorting nature, and (6) with suggestions as to other useful examinations, manipulations, tests, and the like. These two recommendations are covered by a proposed amendment providing for the adoption by the Association, on recommendation by the appropriate referee, of recommended procedures. This term is broad enough to cover all operations where there are impediments to rigid collaborative study, yet whose adequacy has been established by long experience. Such operations would include screening tests to determine whether a precise analysis needs to be made, for making a representative selection of unit packages from a lot, drawing a repre-

* *This Journal*, 31, 63 (1948).

sentative sample from bulk shipments or large units, handling and storing the sample, as well as others of any type designed to guide or facilitate analysis. Not a few of such procedures are of universal application regardless of circumstances or objectives, and we recommend that the 1950 edition of the Book of Methods carry a prefatory note touching on their purpose and proven usefulness, but also pointing out that there may be situations where modifications are called for which, in the judgement and experience of the operator, will better accomplish his purpose.

We recommend further that the title of the Book of Methods be changed to "Official Methods of Analysis of the Association of Official Agricultural Chemists."

PROPOSED AMENDMENTS TO BY-LAWS

(A) Delete paragraphs 5, 6, and 8 and add the following paragraphs:

"5. Methods shall be adopted as 'first action' and 'official.' No method shall be adopted by first action until it has received collaborative study and has been recommended by the appropriate referee at an annual meeting. No method shall be adopted as official except on recommendation of the appropriate referee at an annual meeting following the adoption of such method by first action. No change in an official method shall be adopted until after the changed method has received collaborative study and has been recommended by the appropriate referee at two annual meetings. No official method shall be repealed until such action has been recommended by the appropriate referee at two annual meetings. Such adoption, change, or repeal of methods shall become effective on the thirtieth day after publication of the record of such action in the *Journal* of the Association.

"6. Upon the recommendation by the appropriate referee, the Association may adopt well-established procedures for examination or treatment of a mechanical, microscopic, physical, chemical, or other nature."

W. B. WHITE, *Chairman*

W. F. REINDOLLAR

K. D. JACOBS

Approved.

AMENDMENTS TO CONSTITUTION AND BY-LAWS*

The following amendments to the constitution were approved by vote of the Association, having been presented and read on the first day, at the Monday-morning session.

* The constitution and by-laws reprinted to include the revisions made at the 1948 meeting appear on pages 36-38.

Change Article III by deleting that portion beginning with the word "On," line 19 of the second paragraph, and substituting the following:

"The President and Secretary shall appoint referees, from the active members of the Association, on the general subjects designated by the Committee on Recommendations of Referees, and shall also appoint associate referees on subjects designated by that Committee as supplemental to the general subjects assigned to referees. It shall be the duty of referees and associate referees:

- (1) To direct and conduct research on the methods and subjects assigned;
- (2) To prepare and distribute samples, and direct and conduct collaborative studies of methods; and
- (3) To present at the annual meeting the results of the work done and recommendations for methods to be based thereon."

The amendments to the by-laws proposed in the report of the committee on Classification of Methods were approved by vote of the Association. By-laws 11 and 12 were deleted by action of the Association in the approval of the report of the committee on Definitions of Terms and Interpretation of Results on Fertilizers and Liming Materials.

CONSTITUTION

The Constitution is reprinted to show the changes that were made in 1931, 1934, 1935, 1937, 1941, and 1948.

Article I

NAME AND OBJECT

This Association shall be known as the Association of Official Agricultural Chemists of North America,¹ Inc.

The objects of the Association shall be:

1. To secure, devise, test, and adopt uniform and accurate methods for the analysis of fertilizers, soils, foods, feeding stuffs, dairy products, economic poisons, and other materials relating to agricultural pursuits; also medicinal products; cosmetics; and caustic poisons;
2. To secure uniformity in the statement of analytical results;
3. To conduct, promote, and encourage research in chemistry in its relation to agriculture;
4. To afford opportunity for the discussion of matters of interest to agricultural chemists.

Article II

MEMBERSHIP

Active Members

Chemists and other workers along analytical and research lines in the subjects mentioned in Article I, Section 1, connected with the following institutions of North America, shall alone be eligible *ex officio* to active membership:

1. The United States Department of Agriculture;
2. Any national, state, or provincial experiment station, college, or body engaged in research in agricultural chemistry; and
3. Any national, state, or provincial institution or body charged with official control of any of the materials named in Article I.

Associate Members

Chemists and other workers along analytical and research lines in the subjects mentioned in Article I, Section 1, connected with municipal laboratories in North America charged with control of any of the materials or subjects named in Article I are eligible *ex officio* to associate membership.

Chemists engaged in research in agricultural chemistry who are not eligible to active membership and active members of the Association who lose their right to such membership by retiring from the positions indicated above as requisite for eligibility to active membership may be elected to associate membership upon recommendation of the Executive Committee.

Honorary Members

Upon recommendation of the Executive Committee, persons may be elected to honorary membership by the two-thirds vote of those present at any regular meeting of the Association.

¹ NOTE: The term North America is intended to include the United States and its colonial possessions, Canada, Cuba, the British West Indies, Haiti, Santo Domingo, Mexico, and the republics south of Mexico as far as the Panama Canal. This excludes the French West Indian Islands of Martinique and Guadeloupe.

Article III

OFFICERS AND COMMITTEES

The officers of the Association shall consist of a president, a vice-president, and a secretary who shall also act as treasurer.

These officers shall be elected annually from and by the active members and they shall perform the usual duties of their respective positions. These officers, the immediate past president, and three other active members to be elected by the Association shall constitute the Executive Committee. In case of the inability or disability of any one of the additional members of the Executive Committee, any past president in active membership may be designated to serve in his stead. The special duties of the officers of the Association shall be further defined, when necessary, by the Executive Committee. The elected officers of this Association shall constitute the Board of Directors of the Corporation, With the concurrence of the Executive Committee, the president shall appoint an Editorial Board, and the Executive Committee shall determine the membership and tenure of office of the members of this Board. The secretary-treasurer shall be *ex-officio* chairman and executive officer of the Editorial Board. With the concurrence of the Executive Committee, the president shall appoint a chairman and a committee of twelve other members, which shall be designated a Committee on Recommendations of Referees, one-third of the membership of which shall be appointed at intervals of two years to serve six years, the chairman to be appointed annually. The chairman shall assign the twelve members to subcommittees (A, B, C, and D) and shall assign to each subcommittee the reports and subjects which it shall consider. The President and Secretary shall appoint referees, from the active members of the Association, on the general subjects designated by the Committee on Recommendations of Referees, and shall also appoint associate referees on subjects designated by the Committee as supplemental to the general subjects assigned to referees. It shall be the duty of referees and associate referees:

1. To direct and conduct research on the methods and subjects assigned;
2. To prepare and distribute samples, and direct and conduct collaborative studies of methods; and
3. To present at the annual meeting the results of the work done and recommendations for methods to be based thereon.

Article IV

MEETINGS

The annual meeting of the Association shall be held at such place as shall be decided by the Association, and at such time as shall be decided by the Executive Committee. Announcement thereof shall be made, if possible, three months prior to the time of said meeting. Special meetings shall be called by the Executive Committee when in its judgment it may be necessary.

Article V

CHANGES IN CONSTITUTION

All proposed changes or amendments to this constitution shall be presented in writing and read in full to the Association not later than the second day of the regular annual meeting, shall be referred to the Executive Committee, and after a report from this Committee may be adopted as the first order of business on the third day by a vote of three-fourths of the active members present.

BY-LAWS

1. Any amendment to these by-laws or changes therein may be proposed in the same manner and adopted by the same procedure as amendments to the constitution, but only a two-thirds vote of the active members present shall be required for their adoption.

2. These by-laws or any portion of them may be suspended at any regular meeting of the Association without previous notice, by a vote of three-fourths of the active members present.

3. Only one qualified active member of a department, college, experiment station, board, or other institution shall be entitled to vote on general questions before the whole Association. At the discretion of the Chair, any institutional vote upon which there does not seem to be adequate representation may be conducted by letter ballot.

4. In voting upon questions involving methods of analysis, definitions, nomenclature, and laws or regulations relating to materials mentioned in Article I of the constitution, each of the said institutions shall be entitled to vote only upon questions relating to those materials over which said institution exercises official control.

5. Methods shall be adopted as "first action" and "official." No method shall be adopted by first action until it has received collaborative study and has been recommended by the appropriate referee at an annual meeting. No method shall be adopted as official except on recommendation of the appropriate referee at an annual meeting following the adoption of such method by first action. No change in an official method shall be adopted until after the changed method has received collaborative study and has been recommended by the appropriate referee at two annual meetings. No official method shall be repealed until such action has been recommended by the appropriate referee at two annual meetings. Such adoption, change, or repeal of methods shall become effective on the thirtieth day after publication of the record of such action in the *Journal* of the Association.

6. Upon the recommendation by the appropriate referee, the Association may adopt well established procedures for examination or treatment of a mechanical, microscopic, physical, chemical or other nature.

7. No changes shall be made in the methods of analysis used in official inspection until an opportunity shall have been given all active members having charge of the particular inspection affected to test the proposed changes.

8. When any officer ceases to be eligible for membership in the Association, his position shall be considered vacant, and a successor may be appointed by the Executive Committee to continue in office until the next regular meeting. Should any referee or associate referee resign or cease to be eligible for membership in the Association, his office shall be considered vacant and a successor shall be appointed as prescribed in Article III of the constitution. Should a vacancy occur in the Executive Committee, such vacancy may be filled by the action of the other members.

9. Chemists connected with commercial firms or institutions and others interested in the objectives of the Association who are not eligible to either active or associate membership may attend its meetings, take part in the discussions, and, if permission is obtained from the Executive Committee, may present papers.

REPORT OF THE EDITORIAL BOARD

By H. A. LEPPER, *Chairman*

Since the report at the last meeting, the first printing of 9,000 copies of *Methods of Analysis* has been sold. To meet the continuing demand, an issue of 1,000 copies of an electrolytic reproduction of the book was purchased from the College Offset Press of Philadelphia. The reproduction was bound in green cloth and lettered in gold identically as the original and it is difficult, even on close scrutiny, to detect any distinction between the two. A second reissue of 1,000 copies followed the sale of the first, and as of September 30 we had 450 copies on hand. It is hardly likely that this demand will be maintained with the approach of the publication of the seventh edition in 1950. During the coming year a new committee on revision of methods must be appointed to undertake the preparation of the seventh edition immediately after the 1949 meeting. This year will require more than the usual activity on the part of the referees to bring the respective chapters into conformity with the newly adopted classification of methods.

The financial status of our *Journal* will be discussed in the Secretary's report. Dr. White will present the Editor's report on the *Journal*.

Approved.

REPORT OF THE EDITORIAL COMMITTEE OF THE *JOURNAL*

W. B. WHITE, *Editor and Chairman*

Again there were annoying delays in the printing of the *Journal*, due to production difficulties, so that all issues were late in getting to our subscribers. At the time this report was prepared we did not have the page proof of the November issue, and thus must estimate our increase in numbers of pages by the first three issues only. Last year there were 594 pages; this year 800 pages.

In other respects we can measure our growth for the entire year. Last year there were 38 contributed papers, 3 notes, and 7 book reviews; this year there will be 43 papers, 3 notes and 9 book reviews.

Despite the unsettled conditions abroad, involving shortage of dollar exchange and even stoppage of the mails, there was a gratifying increase in the number of subscribers from 1800 to 1917. You will note, however, from the Treasurer's report that the *Journal* is very far from being self-supporting. All members are urged to take every opportunity to point out to educational institutions, experimental stations, and others the value of the *Journal* as a source of up-to-date regulatory methods and as a record

of the genesis and testing of new methods. The committee appointed to publicize the value of the *Journal* to potential subscribers will welcome suggestions along this line as to advertising media, lists of prospects, and the like. These may be sent to W. A. Queen, H. A. Lepper, or W. B. White, all of the U. S. Food and Drug Administration, Washington 25, D. C.

Again we are indebted to our contributors for the high caliber of the papers submitted, and for their unfailing courtesy in considering editorial suggestions. Our able reviewing staff has been invaluable in reaching decisions on what papers would be of interest to our readers, what revisions should be made in manuscripts, and what book reviews should be published. The Committee takes this opportunity of extending thanks both to contributors and reviewers.

Approved.

REPORT OF COMMITTEE TO CONFER WITH AMERICAN PUBLIC HEALTH ASSOCIATION ON STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS

Your Chairman is happy to report that copies of the Ninth Edition of *Standard Methods for the Examination of Dairy Products* became available late in August. The introduction of a simple cross reference system for the thirteen chapters and the carefully organized arrangement of the applications and interpretations of quality tests in Chapter 1 makes this edition of *Standard Methods* distinctively more useful than previous editions both to administrators and to laboratory workers.

In order that certain laboratories engaged in the examination of milk and cream, by one or more of the four routine methods described, may have several copies of the directions in which they are most interested without buying the complete edition, paper bound separates of Chapter 2 will be available. Chapter 2 describes the Agar Plate Method, the Direct Microscopic Method, the Methylene Blue Reduction Method, and the Resazurin Reduction Method. Many teachers of dairy sanitation will want their students to have copies of the separate as a reminder after graduation that there is a *Standard Methods*, and an American Public Health Association.

In addition to the separate referred to above, a "cook-book" style revision of Chapter 2 is in process. The intent is that this style of presentation will provide teachers of bacteriology with an abridged laboratory guide, which will in turn establish greater uniformity of instruction among students.

Action taken by this Association in 1947 relative to the deletion of all previously recognized tests for residual phosphatase in heat treated dairy products, and the adoption in place thereof of the Sanders and Sager method, has created a problem. In the Ninth Edition of *Standard Meth-*

ods, description of the Sanders and Sager method is limited to its application to hard type cheese. The New York State Department of Health method and the New York City Department of Health Laboratory Method No. 1 have been included among the official procedures for application to milk.

Soon after the Boston meeting it is expected that the details of the complete Sanders and Sager procedure will be printed in the American Journal of Public Health. Reprints of this procedure will then be made available to all laboratories who wish to compare the Sanders and Sager procedure with the methods which have been officially recognized in the Ninth Edition of *Standard Methods for the Examination of Dairy Products*. Before the next edition of *Methods of Analysis* is printed, it is hoped that the differences can be reconciled.

A. H. ROBERTSON, *Chairman*
GUY G. FRARY
J. O. CLARKE

Approved.

REPORT OF COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

The following definition was proposed at the last meeting and should be made tentative.

Fused Calcium-Magnesium Phosphate is a product derived from the fusion of rock phosphate with approximately thirty per cent (30%) of magnesium oxide (MgO), as such or as a mineral silicate. Its fineness and content of available phosphoric acid (P_2O_5) shall be stipulated. Example: Fused Calcium-Magnesium phosphate, twenty per cent (20%) available phosphoric acid (P_2O_5).

It is the unanimous vote of the Committee that the work on Definition of Terms and Interpretation of Results on Fertilizers and Liming Materials be discontinued and the Committee be discharged. Such action obviates the need for by-laws 11 and 12, and their deletion is recommended.

Approved.

L. S. WALKER, *Chairman*
F. W. QUACKENBUSH
M. ELMER CHRISTENSEN
L. E. BOPST
JOHN B. SMITH
W. H. MACINTIRE

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

WM. F. REINDOLLAR, *Chairman*

In the 1947 report of this Committee a plan to simplify the handling and distribution of referee reports and to relieve referees and associate referees of the requirement of sending reports to several individuals was recommended. This proposal, in brief, was to have each associate referee mail six copies of his report to the Secretary-Treasurer who in turn forwarded them to the chairman of the committee, the general referee, and the members of the appropriate subcommittee. A similar system applied to general referees except that they send five copies to the Secretary-Treasurer and retain one for themselves. This plan was put into operation during the past year and appears to be generally satisfactory. Early in the year referees and associate referees were sent an abstract of the 1947 Committee report together with the "Guiding Considerations Relative to Collaborative Work." This was followed by another letter in June, again stressing the new procedure for handling reports, and a copy of the report of the Committee on the Classification of Methods. Finally, each member of the group received a return postcard asking whether or not he would have a report and advising him when it was due. As a result of these efforts, a majority of the reports were sent to the proper source, the Association's Secretary was informed regarding papers to be presented, and the formulation of the program materially facilitated.

The chairman wishes to express his appreciation to the several subcommittees, referees, associate referees, and collaborators whose efforts have contributed so materially to the success of this Conference.

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

By H. A. HALVORSON, Department of Agriculture, Dairy and Foods,
St. Paul, Minn., *Chairman*: E. L. GRIFFIN, and J. B. SMITH

FEEDING STUFFS

It is recommended—

(1) That further study be made on the following:

- (a) Calcium and iodine in mineral mixed feeds.
- (b) Lactose in mixed feeds.
- (c) Adulteration of condensed milk products.
- (d) Crude fat or ether extract.

* These recommendations submitted by Subcommittee A were approved by the Association. Unless otherwise given all references are to *Methods of Analysis*, A.O.A.C., 1945.

- (e) Fluorine.
- (f) Protein evaluations in fish and animal products.
- (g) Hydrocyanic acid glucosides.
- (h) Sampling and analysis of condensed buttermilk.
- (i) Microscopic examination of feeds.
- (j) Tankage (hide, hoof, horn, and hair content).

(2) That the tentative method for calcium and phosphorus, *This Journal* 31, 98 (1948), be made official, first action.

(3) That the tentative acetone method for fat in fish meal, *This Journal*, 31, 98 (1948), be adopted as official, first action.

FERTILIZERS

It is recommended—

(1) That study of sampling equipment and method of sampling be continued.

(2) That preparation of sample for analysis be studied.

(3) That the formaldehyde titration method, as recommended by the Associate Referee be adopted as official, first action for determining nitrogen in ammonium nitrate.

(4) That the study of determining nitrogen in high nitrate-chloride mixtures be continued.

(5) That a survey be made of the different types of mills being used for the preparation of samples.

(6) That collaborative work on determining potash in samples prepared by different mills be continued on a greater variety of samples.

(7) That the changes in methods for phosphoric acid described in the first, second, third, and fourth recommendations of the Associate Referee be made.

(8) That sec. 2.16 be altered (first action) by

(a) Changing the phrase, in sec. 2.16(a) first line, "*Acidulated samples*" to "*Acidulated samples and mixed fertilizers*."

(b) Deleting the words, in sec. 2.16(b) first line, "*other than basic slag*."

(9) That sec. 2.17 be changed by deleting the words, in second sentence, "in acidulated samples, dicalcium phosphate, precipitated bone phosphate and precipitated bone" (first action).

(10) That the methods for citric acid-soluble phosphoric acid in basic slag, sec. 2.18, 2.19, and 2.20, be deleted (first action).

(11) That work on methods for phosphoric acid be continued, with emphasis on

(a) Evaluation of sintered, fused, and calcined alpha phosphates as fertilizers.

(b) Aging of the molybdate solution used in the volumetric method to determine if a time limit should be put on its use or an addition made to preserve it.

(12) That the air-flow method be made official for determining free water in fertilizers, first action.

(13) That the vacuum-desiccation method with a drying period of 16 to 18 hours be made official for determining free water in fertilizers, first action.

(14) That the official procedure for oven drying be modified to state only one drying temperature, the selection of the temperature to be determined by study, not necessarily collaborative, during the coming year.

(15) That further study be made on the applicability of the fore-mentioned three methods.

(16) That the tentative method for determining moisture in fertilizers with the use of distillation with toluene be deleted from the "Methods of Analysis."

(17) That the phraseology of the present official procedure for oven drying be modified as follows:

(a) Change parenthetical remark now worded "Not applicable to samples containing compounds other than H_2O that are volatilized at the temp. of drying." to read "Not applicable to samples that yield volatile substances other than H_2O at the temp. of drying."

(b) Change first sentence of text now worded "Heat 2 g of prepared sample, 2.2, for 5 hours in water oven at temp. of boiling H_2O (98–100°) to read "Heat 2 g of prepared sample, 2.2, for 5 hours in oven at temp. of 99–101°"

(c) Change second sentence of text now worded "In case of potash salts, $NaNO_3$, and $(NH_4)_2SO_4$, heat at ca 130° to constant weight." to read "In case of $NaNO_3$, $(NH_4)_2SO_4$, and potash salts heat to constant weight in oven at temp. of 129–131°."

(d) Change section heading to read *Water* instead of *Moisture*.

(18) That the work of the other Associate Referees on fertilizers be continued.

ECONOMIC POISONS

It is recommended—

(1) That a study be made of the determinations of rotenone in the presence of other insecticidal and fungicidal ingredients or of diluents.

(2) That work be continued on the analysis of oil emulsions that are prepared with non-soap emulsifiers, giving consideration to the use of chromatography for determination of the oil.

(3) That work on methods for the analysis of rodenticides containing alpha naphthyl thiourea (ANTU) and sodium fluoroacetate (1080) be continued.

(4) That method No. 20 for the determination of 2, 4-dichlorophenoxyacetic acid, and method No. 21 for the determination of salts of 2, 4-dichlorophenoxyacetic acid as described in the report of the Associate Referee be continued, giving consideration to the amount and manner

of adding the indicator so as to improve the end-point in the titration.

(5) That method No. 23, as described in the report of the Associate Referee for determination of esters of 2, 4-dichlorophenoxyacetic acid in the presence of soap, acids, alcohols, and oils, be further studied with emphasis placed upon a broader survey of ester mixtures and upon methods of breaking emulsions formed by this type of product.

(6) That the study of methods for the analysis of tetraethyl pyrophosphate preparations be continued.

(7) That the methods for determination of DDT, based on determination of the total chlorine content, which were adopted as tentative last year, be adopted as official methods, first action.

(8) That work on the determination of benzene hexachloride be continued.

(9) That study be started on organic thiocyanates and dimethyl dithio carbonate.

DISINFECTANTS

The Referee and his Associates have presented before the Association two papers relative to semi-micro methods of testing disinfectants and factors affecting the resistance of test organism. It is recommended that the work be continued.

LEATHER AND TANNING MATERIALS

No report was received. It is recommended that these chapters be dropped and the work be discontinued.

PLANTS

It is recommended—

(1) That the study of methods for zinc in plants be continued.

(2) That the magnesium uranyl acetate method for sodium in plants be kept tentative and that the study be continued, especially in respect to tolerance of the procedure to various levels of interfering ions which occur in plant materials.

(3) That collaborative work on the lignin method be continued for another year, using other samples of plant material.

(4) That the cellulose method be studied to determine the causes for the variations in results which have been found.

(5) That the spectrophotometric measurement of starch-iodine dispersions be investigated as a possible method for the determination of starch in samples low in starch.

(6) That the "Chromotrope B" method for boron be further investigated and that collaborative work on it be undertaken this year.

(7) That the other Associate Referees retain their assignments and continue their studies.

SPECTROGRAPHIC METHODS

No report has been received. It is recommended that work on this subject be continued.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That studies on the "combination dithizone-spectrographic method" and on the polarographic procedure for the determination of zinc in soils be continued.

(2) That the study of the determination of copper in soils be continued.

(3) That the utilization of carmin as an indicator in the determination of boron content of soils be studied further and that p-nitrobenzeneazo-1, 8-dihydroxy naphthalene-3, 6-disulphonic acid, or "chromotrope-B" be studied as a suitable reagent for the determination of boron in soils.

(4) That further studies of pH in soils of arid and semi-arid regions be based upon soil systems of moisture content representative of an air-dry soil.

(5) That the analytical technic previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.

(6) That a study be made as to the adequacy of calcium hydroxide as a fixative for fluorine in soil charges of 1 to 1 proportion with calcination at 500°C. in 5 to 60 minute periods.

(7) That the direct distillation of unignited soil with H₂SO₄ at 165°C., followed by distillation of an aliquot at 135°C. be studied collaboratively.

(8) That the "2-point" barium hydroxide-barium acetate titration procedure for the determination of exchangeable hydrogen in soils, as reported upon at this meeting, be studied further in relation to calcite equilibria in a variety of soils.

(9) That the survey and comparison of methods for the determination of phosphorus, (a) that fraction in "available" state and (b) the proportions of organic-inorganic forms therein, be continued (*This Journal*, 30, 43).

(10) That the survey and comparisons of methods for the determination of exchangeable potassium in soils be continued (*This Journal*, 30, 44).

(11) That the tentative procedures for neutralization value of calcium silicate slags, 3.11(a), be annotated by the statement "without correction for sulphide content." This constitutes a clarification of (13), *This Journal*, 31, 44.

(12) That the procedure for the determination of sulfide sulfur content of calcium silicate slags, as reported by the Associate Referee, be adopted as tentative.

(13) That the Associate Refereeship on Exchangeable Calcium and Magnesium be maintained.

STANDARD SOLUTIONS

It is recommended—

(1) That method II for titanium trichloride solutions, 21.37 (p. 290),

substituting potassium dichromate for potassium permanganate be made official, first action.

(2) That preparation and standardization of potassium dichromate solutions be studied further, giving consideration to at least one recrystallization of potassium dichromate to insure uniformity.

(3) That the method of standardization of hydrochloric acid with standard sodium hydroxide (which was adopted as official, first action, in 1938) be adopted as official, final action. (A.O.A.C. Methods 43.7 and 43.8).

(4) That the method for standardization of sulfuric acid by the standard borax method (which was adopted as tentative in 1939) be adopted as official, first action. (A.O.A.C. Methods 43.14 and 43.15)

VITAMINS

It is recommended—

(1) That, with the following minor corrections, the present tentative method for vitamin A in fish liver oils be made official, first action:

The ether in 36.2 (page 599), should be specified as "U.S.P. ethyl ether in $\frac{1}{2}$ pound cans, anesthesia grade, free from peroxides."

In the second sentence of 36.3 the "ground glass joint" should be changed to "glass joint." In the seventh line of 36.3, change of the time from "2 minutes" to "allow the mixture to stand (about 2 minuter) until separation is visible complete, as determined by the absence of refraction streaming and the presence of distinct layers." In 36.4 (first line, p. 600) change "0.4" to "0.398." In 36.6 (p. 601) density values in the table are to be corrected to agree with the following—

T.	D.
0.7	2.16
7.0	1.16
11.0	0.959
26.5	0.577
27.5	0.561
29.5	0.530
32.5	0.488
55.5	0.256
63.0	0.201
71.5	0.146

(2) That the growth method for thiamine (vitamin B₁), (36.16–36.23), be made official, first action.

(3) That the fermentation method for thiamine (36.27–36.31) be made official, first action.

(4) That the fluorometric (thiochrome) method for thiamine (36.24–36.26) be changed as recommended in the report of the Associate Referee

and that it remain official, first action, and be studied further during the coming year.

(5) That the tentative microbiological method for the determination of riboflavin (*This Journal*, 30, 79) be dropped and the directions as revised by the Associate Referee be made official, first action.

(6) That the fluorometric method for the assay of riboflavin, as described by the Associate Referee, be made official, first action.

(7) That the method for vitamin C (36.47-36.48) be revised to include the minor corrections and additions suggested by the Associate Referee and that it then be made official, final action.

(8) That the tentative method for determining vitamin D in milk (36.49-36.60) be made official, first action.

(9) That the Associate Referee on folic acid, on the basis of this year's results, select a single procedure and subject it to intensive collaborative study during the coming year.

(10) That the alternative tentative method (*This Journal*, 31, 111, 1948) be adopted as official, first action, for carotene in hays and dried plants.

(11) That the method for carotene (*This Journal* 30, 84, 1947) be discontinued for hays and dried plants but that it be continued as a tentative method of analysis for other materials.

(12) That the official, first action, method, *This Journal*, 30, 82-84 (1947), for nicotinic acid be revised as recommended by the Associate Referee.

(13) That studies on the analysis of carotene be continued.

(14) That the work of the Associate Referees on other vitamins be continued.

(15) That the method for vitamin A in animal feeds be studied.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

By H. J. FISHER (Agricultural Experiment Station, New Haven, Connecticut), *Chairman*; G. R. CLARK, and F. H. WILEY

NAVAL STORES

No report was received. It is recommended that the subject be continued.

RADIOACTIVITY

No report was received. It is recommended that the subject be continued.

* These recommendations submitted by Subcommittee B were approved by the Association. Unless otherwise given all references are to *Methods of Analysis*, A.O.A.C., 1945.

VEGETABLE DRUGS AND THEIR DERIVATIVES

It is recommended—

- (1) That the study of chemical methods for ergot alkaloids be discontinued.
- (2) That the proposed method for physostigmine in ointments be adopted as official, first action, and the topic closed.
- (3) That the subject of theobromine and phenobarbital be studied further.
- (4) That the study of the separation of aminopyrine, ephedrine, and phenobarbital be reassigned.
- (5) That the subject of quinine be continued for the purpose of submitting the Herd procedure to collaborative study to determine if it should be made official.
- (6) That the subject of chemical methods for penicillin be dropped.
- (7) That the subject of rutin in tablets be continued.
- (8) That the subject of ethylmorphine in syrups be continued.
- (9) That method 39.73 for arecoline hydrobromide be made official (first action).

SYNTHETIC DRUGS

It is recommended—

- (1) That the subject of methylene blue be continued.
- (2) That the subject of sulfanilamide derivatives be continued.
- (3) That the subject of propadrine hydrochloride be continued.
- (4) That the subject of carbromal be continued.
- (5) That the subject of butacaine sulfate be continued.
- (6) That the subject of spectrophotometric methods be continued.
- (7) That the proposed method for trichlorethylene be adopted as official (first action), and the subject be closed.
- (8) That the subject of propyl thiouracil be reassigned.
- (9) That the subject of phenolphthalein in chocolate preparations be continued.
- (10) That the subject of pyribenzamine and benadryl be continued.
- (11) That the subject of synthetic estrogens be reassigned.

MISCELLANEOUS DRUGS

It is recommended—

- (1) That the following topics on which no reports have been received be continued:
 - Microscopic tests for alkaloids and synthetics
 - Alkali metals
 - Glycols and related compounds
 - Preservatives and bacteriostatic agents in ampul solutions
 - Estrone and estradiol

- (2) That the subject of chromatographic separation of drugs be dropped.
- (3) That a collaborative study be made of the application of the Rotondaro method to drugs containing mercury as a major ingredient.
- (4) That the subject of the separation of bromides, chlorides, and iodides be continued.
- (5) That the proposed method for calcium, phosphorus and iron in vitamin preparations be adopted as official (first action) and the subject closed.
- (6) That method 39.202 for iodine be reworded as recommended by the Associate Referee (first action).
- (7) That the subject of methyl alcohol be continued for the specific purpose of restudying the accuracy of method 39.162.

COSMETICS

It is recommended—

- (1) That the following topics be continued:
 - Cosmetic creams
 - Deodorants and anti-perspirants
 - Depilatories
 - Hair dyes and rinses
 - Moisture in cosmetics
 - Mascaras, eyebrow pencils, and eye shadow
 - Cosmetic skin lotions
- (2) That the following topics be discontinued:
 - Alkalies in cuticle removers
 - Mercury salts in cosmetics
 - Hair straighteners
- (3) That the proposed methods for pyrogallol in hair dyes be adopted as official, first action, and the topic closed.
- (4) That the proposed methods for the analysis of face powder be adopted as official, first action, with the following changes:
 - (a) That the methods be adopted individually, as methods for the various constituents of face powder rather than as an entire method for the analysis of face powder. This will provide for the addition of methods for other constituents, not included in the study to date, should the necessity for them appear.
 - (b) The proposed method for stearate be designated "Fats and Fatty Acids as Stearic Acid."
- (5) That the subject of sun tan preparations be studied.

COAL-TAR COLORS

It is recommended—

- (1) That the following topics be continued:
 - Acetates, carbonates, halides, and sulfates in coal-tar colors

Buffers and solvents in titanium trichloride titration
Ether extract in coal-tar colors
Identification of coal-tar colors
Volatile amine intermediates
Non-volatile unsulfonated amine intermediates in coal-tar colors
Sulfonated amine intermediates in coal-tar colors
Unsulfonated phenolic intermediates in coal-tar colors
Sulfonated phenolic intermediates in coal-tar colors
Intermediates derived from phthalic acid
Mixtures of coal-tar colors for drug and cosmetic use
Spectrophotometric testing of coal-tar colors
Subsidiary dyes in D&C colors
Subsidiary dyes in FD&C colors
Hygroscopic properties of coal-tar colors

(2) That the proposed method for the determination of pure dye in D&C Reds Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 31, and 34 and Ext. D&C Red No. 2 be adopted as official (first action) and the subject continued.

(3) That the proposed method for lead in coal-tar colors not containing calcium, barium, or strontium be adopted as official (first action), and the subject closed.

(4) That the proposed method for lead in lakes of coal-tar colors be adopted as official (first action), and the subject closed.

(5) That the following new topics be studied:

Arsenic in coal-tar colors

Heavy metals in coal-tar colors

(6) That the boiling range of pseudocumidine and xylidine in certified coal-tar colors be studied.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By J. O. CLARKE, Food and Drug Administration, Washington,
D. C., *Chairman*; PAUL A. CLIFFORD; and A. H. ROBERTSON

PROCESSED VEGETABLE PRODUCTS

It is recommended—

(1) That studies of methods for determining quality factors in canned and frozen fruits and vegetables be continued.

(2) That studies of the determination of moisture in dried vegetables be continued.

* These recommendations submitted by Subcommittee C were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

(3) That studies of methods for the estimation of enzymatic activity of frozen fruits and vegetables be continued.

FILL OF CONTAINER METHODS

It is recommended—

(1) That studies of methods for determining fill of container for foods, drugs, and cosmetics be continued.

COFFEE AND TEA

It is recommended—

(1) That a Referee be appointed to review the chapter on coffee and tea and make recommendations for additional work where this appears desirable.

(2) That the Fendler-Stüber method (Modified; 18.15) be made official, final action.

COLORING MATTERS IN FOODS

It is recommended—

(1) That the rapid method of detection of small amounts of tartrazine (FD&C Yellow No. 5), as published in *Methods of Analysis*, 20.125, be made official, final action with slight modification adopted last year, *This Journal*, 31, 82 (1948).

(2) That the method outlined by the Referee for the quantitative estimation of FD&C Yellow No. 5 (tartrazine) in the presence of FD&C Yellow No. 6 (Sunset Yellow F.C.F.) be tested collaboratively.

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green S.F. Yellowish), FD&C Green No. 3 (Fast Green F.C.F.), and FD&C Blue No. 1 (Brilliant Blue F.C.F.).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow AB), FD&C Yellow No. 4 (Yellow OB), FD&C Orange No. 2 (Orange SS), and FD&C Red No. 32 (Oil Red XO).

(5) That investigational work on analytical methods for coal-tar colors certifiable for use in foods be conducted.

DAIRY PRODUCTS

It is recommended—

(1) That the Sanders-Sager method for phosphatase in dairy products be further studied; and that the Associate Referee conduct collaborative work on milk or cream, comparing results by this method with those of the phosphatase tests given in 22.43 to 22.57.

(2) That methods for the determination of ash in milk and in evaporated milk be further studied.

(3) That the statement in the method for fat in cheese, 22.130, "Add ca 0.5 g of sand, previously digested with HCl, to prevent bumping . . ." be changed to read, "Add a few glass beads or other inert material, pre-

viously digested with HCl, to prevent bumping, . . ."; and that study of methods for sampling, fat, and moisture in cheese be continued.

(4) That studies of methods for the detection of chlorine added to milk be discontinued.

(5) That studies of methods for the determination of the acidity of milk be continued.

(6) That the use of mechanical stirring or shaking devices and of artificial methods of cooling, suitable for use in the present official method for the preparation of butter samples, be further studied.

(7) That studies of methods for the detection of reconstituted milks be continued.

(8) That the Associate Referee conduct fundamental studies of the acetic serum method (22.28) and the copper serum method (22.30); and that the sour serum method (22.29) be dropped, first action.

(9) That studies be continued to ascertain whether the present Babcock method for fat in milk should be modified when used for the determination of fat in homogenized milk; and that the Roesse-Gottlieb method for fat in dairy products be further studied.

(10) That studies be undertaken on sampling and preparation of sample of soft cheese.

(11) That studies be continued on methods for frozen desserts.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That an Associate Referee be appointed to study the present tentative method for fat by acid hydrolysis (23.8).

(2) That the Referee study paragraphs 23.29 and 23.30 (Acidity of Ether Extract, Official) and 23.31 and 23.32 (Tentative, Rapid Method) with a view towards formulation of a consolidated procedure retaining the best features of each, and that collaborative studies be undertaken.

(3) That the tentative qualitative method for glycerol (23.25), and the quantitative method as outlined by the Associate Referee, be tested collaboratively with a view towards official adoption next year.

(4) That an Associate Referee be appointed to investigate the value of the method for ammonia nitrogen (23.33) in the estimation of the age of shell eggs.

MICROANALYTICAL METHODS FOR EXTRANEEOUS MATERIALS IN FOODS AND DRUGS

It is recommended—

(1) That the method for rodent excreta, in corn meal, 42.32, be made official final action.

(2) That the methods for mold in cranberry sauces, *This Journal*, 31, 783 (1948), for fruit products and beverage materials be adopted as tentative, and that the suggested methods for strawberries be further studied.

(3) That the method for extraneous materials in starch, 42.38, as modified by the Associate Referee, be adopted as tentative; that work on flour, 42.29-42.31, be continued; that the suggested methods for filth in popcorn be made tentative; and that collaborative work on baked products and prepared cereals be undertaken.

(4) That the new methods for pepper and prepared mustard, as devised by the Associate Referee, be subjected to collaborative study.

(5) That collaborative studies on filth in nuts and nut products, confectionery, and eggs and egg products be undertaken.

(6) That the wording of 42.61 (b) and 42.75 be changed as recommended by the Associate Referee.

SEDIMENT TEST (MILK AND CREAM)

It is recommended—

(1) That the method outlined by the Referee for the preparation of standard sediment discs be subjected to collaborative study.

(2) That further work be done on the preparation and use of standard sediment discs rendered permanent by treatment with a transparent plastic or by other means.

(3) That this topic be properly assigned to the chapter "Extraneous Materials in Foods and Drugs."

DECOMPOSITION IN FOODS

It is recommended—

(1) That the revised method for the study of volatile acids in fish be studied collaboratively.

(2) That the method for water-insoluble acids in butter and cream be adopted as official, first action.

(3) That the study of chemical means for detecting decomposition in fruits, in particular, "blackheart" in pineapple, be developed further.

(4) That the study of chemical means for detection of decomposition in dairy products be continued.

(5) That an Associate Referee be appointed to study succinic acid and water-insoluble acids as indices of decomposition in eggs and egg products and in fish.

(6) That the title of this topic be revised to: "Chemical Indices of Filth and Decomposition in Foods."

GELATINE, DESSERT PREPARATIONS, AND MIXES

It is recommended—

(1) That work in this field be continued, as recommended by the Referee.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the extract obtained by using a mixture of ethyl ether and

petroleum ether, *This Journal*, 31, 334 (1948), be designated "Crude Fat (Acid Hydrolysis Method)" and that the method so designated be adopted as official, first action.

(2) That work be continued on methods for the determination of total solids in fish.

(3) That a study be made of methods for the determination of drained liquid in clams.

GUMS IN FOOD

It is recommended—

(1) That the method for detection of gums in mayonnaise and French dressing, 33.57, be amended by substituting "50 ml" for "1.5 oz." in line 3, paragraph 2, and that the method, as amended, be adopted as official, first action.

(2) That studies be continued on the detection of gums in soft curd cheeses.

(3) That studies be continued on the detection of gums in cacao products.

(4) That studies be continued on the detection of gums and other stabilizers in frozen desserts.

(5) That an Associate Referee be appointed to study detection of gums in catsup and related tomato products.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That work be continued for the development of methods for the determination of soya flour in meat products.

(2) That work be initiated on the development of a method for the determination of non-fat dry milk solids in meat products.

(3) That studies be made of additional methods for the determination of creatin in meat products.

(4) That methods for the detection of horse-meat in ground meat be developed and that an Associate Referee be appointed for this purpose.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

It is recommended—

(1) That the study of methods for the determination of cadmium, copper, lead, mercury, and zinc in foods be continued with collaboration where necessary.

(2) That the study on the determination of DDT in foods in general be continued with sufficient collaboration to support adoption and inclusion of a DDT method in the next edition of *Methods of Analysis*.

(3) That the effect of the canning process on the decomposition of the newer insecticides be studied, with respect to the nature of possible decomposition products and their effects on their methods of analysis.

(4) That a new Associate Referee on the determination of parathion be appointed for study of that subject.

MICROBIOLOGICAL METHODS

It is recommended—

(1) That work be continued on canned meats, canned acid foods, canned vegetables, eggs and egg products, nuts and nut products, frozen fruits and vegetables, sugar, and canned fish.

MICROCHEMICAL METHODS

It is recommended—

(1) That a collaborative study of microchemical methods for carbon, hydrogen, and nitrogen be undertaken.

(2) That the chapter be expanded by the inclusion of other valuable methods of elemental microanalysis.

NUTS AND NUT PRODUCTS

It is recommended—

(1) That methods suggested by the Referee for the microscopic examination of nuts and nut products be referred to the Referee on Chapter 42 of the *Methods of Analysis*.

(2) That the methods for preservation and preparation of sample be adopted as tentative.

(3) That methods for moisture, crude fat, crude protein, crude fiber, ash, reducing sugars, sucrose, and salt, as suggested by the Referee, be adopted as tentative and further studied with a view towards their official adoption.

(4) That paragraphs 30.1 through 30.10, 30.13 through 30.17, be dropped. (Recommendation 3, above, substitutes more suitable methods.)

(5) That methods for added coloring materials, preservatives and artificial sweeteners, and metals, other elements, and residues, be adopted as tentative by reference to the appropriate chapter.

(6) That sorting methods for moisture and fat, and methods for added starch in nut butters and pastes, and added glycerol and glycols be studied.

OILS, FATS, AND WAXES

It is recommended—

(1) That a chromatographic procedure for the purification of unsaponifiable matter be further studied.

(2) That the modified Bellier Test, 31.47–31.48, as revised be adopted as official, first action, and further efforts be directed towards making the test quantitative.

(3) That studies of methods for the determination of antioxidants in fats be continued.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That work on the esters of benzoic and vanillic acids be discontinued for the present.

(2) That methods for the quantitative determination of saccharin be further studied and that the method described by the Associate Referee, *This Journal*, 30, 492 (1947), be tested collaboratively.

(3) That the ferricyanide method for quaternary ammonium compounds in commercial preservatives, *This Journal* 31, 105 (1948), be made official, final action; that the method for table sirup, *ibid.*, 31, 108, be made official, final action; that the method for bottled beverages containing fruit juice, *ibid.*, 31, 106, be made official, final action; that the method for beer, *ibid.*, 31, 108, be made official, final action.

That collaborative study be continued on methods for the determination of quaternary ammonium compounds in fruit juices, bottled sodas, milk, mayonnaise, salad dressings, and sandwich spreads and pickles and relishes.

(4) That the method for the determination of monochloroacetic acid in carbonated beverages, *This Journal*, 31, 104, with the changes suggested by the Associate Referee, be adopted as official, final action.

That the method for the determination of monochloroacetic acid in beer and wine, with the changes suggested by the Associate Referee, be made official, first action.

That the method for monochloroacetic acid in commercial preservatives, as recommended by the Associate Referee, be made official, first action.

That the barium test, barium-indigo test, indigo test, and pyridine tests for qualitative identification of monochloroacetic acid in commercial preservatives be adopted as official, first action.

That the qualitative indigo test and pyridine tests for monochloroacetic acid in carbonated beverages, orange juice, beer, and wine be adopted as official, first action.

That further work be done on fruit juices containing orange juice.

That further work be done on qualitative tests for monochloroacetic acid.

(5) That work on dichloroacetic acid be discontinued for the present.

(6) That work be continued on the development of improved methods for the detection of formaldehyde in foods.

That the modified methods selected by the Associate Referee be submitted to collaborative study and that unnecessary and unused methods be deleted.

(7) That use of an alternative chromatographic technique for the identification and estimation of volatile acids in baked products be further investigated.

(8) That the rapid oxidation method for thiourea in oranges and orange juice outlined by the Associate Referee be adopted as tentative and that the present tentative method for thiourea in orange juice be dropped.

That work on the determination of thiourea in foods be continued.

(9) That work be continued on methods for the estimation of dulcin in foods.

That work be initiated on methods for the detection and estimation of the artificial sweetener having the composition, propoxy, 2-amino, 4-nitrobenzene.

SPICES AND OTHER CONDIMENTS

It is recommended—

(1) That studies of methods for the detection of caramel in vinegar be continued.

(2) That studies of the determination of tartrates in vinegar be continued.

(3) That the permanganate oxidation method, 33.91, as modified by the Associate Referee, be made official, first action.

(4) That methods for the determination of free mineral acids in vinegar be further studied.

(5) That methods for determining sorbitol, and the usefulness of this value in detecting cider vinegar in wine vinegar, be studied.

(6) That the official, first action, method for starch in mayonnaise and salad dressing be made official, final action.

(7) That work be carried out looking toward the adoption, as official, of methods for the preparation of samples and determination of total fat in mayonnaise and salad dressing, particularly as regards determination of fat in starchy salad dressings low in fat.

(8) That the efficiency of the preliminary removal of fat in the official method for nitrogen in mayonnaise be studied.

(9) That the official, first action, method for starch in prepared mustard and mustard flour be made official final action.

(10) That studies be initiated looking toward adoption of a method for the determination of ash in prepared mustard.

(11) That studies be continued on the determination of sugars in prepared mustard.

(12) That studies be continued of methods for the determination of volatile oils and other pungent principles in prepared mustard and mustard flour.

(13) That studies be continued for methods for the determination of volatile oil in spices.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

By C. S. FERGUSON, State Department of Public Health, Boston, Mass.,
Chairman; KENNETH L. MILSTEAD, AND J. WALTER SALE.

ALCOHOLIC BEVERAGES

Malt Beverages, Brewing Materials, and Allied Products:

It is recommended—

- (1) That study of methods for determination of essential oil and resins in hops be continued.
- (2) That the dye color method for the estimation of color in wort and beer, described in the Associate Referee's report for 1948, be adopted as official, first action.
- (3) That the tentative method for color in beer and wort, *This Journal*, 30, 68 (1947), be dropped.
- (4) That work on photometric beer color evaluations be continued.
- (5) That study of beer turbidity methods be continued.
- (6) That the tentative method, 14.112–14.115, for total solids in yeast, be adopted as official, first action, including the revision of 14.114, preparation of sample, for total solids as described in the report of the Associate Referee.
- (7) That the Milos test for caramel, 14.35, be deleted, final action.
- (8) That the Mathers test for caramel, *This Journal*, 31, 76 (1948), be studied collaboratively with respect to its application to beer.
- (9) That the study of carbon dioxide in beer be continued.
- (10) That the direct (non-ashing) orthophenanthroline method, described in Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, pages 32 and 37, for the determination of iron in beer, be studied further (a) with a view to eliminating the use of the reducing agent, hydroxylamine hydrochloride, and (b) use of crystalline ferrous ammonium sulfate in place of metallic iron for standardization.
- (11) That further work on copper be postponed, pending outcome of proposed work by the Referee on metals in foods.
- (12) That work be continued on polarographic and spectrographic methods for tin in beer.

Wines:

It is recommended—

- (1) That chromatographic studies of wine be continued.

* These recommendations of Subcommittee D were approved by the Association. Unless otherwise given all references are to *Methods of Analysis*, A.O.A.C., 1945.

(2) That the official Milos test for caramel (15.38) be deleted, final action.

(3) That the official, first action, Mathers test, *This Journal*, 31, 76 (1948), be adopted as official, final action.

Distilled Liquors:

It is recommended—

(1) That the study of methods of analysis with reference to the aging or maturing of whisky in laminated (plywood) barrels be continued.

(2) That the Mathers test for caramel, *This Journal*, 31, 76 (1948), be adopted as official, final action, for distilled liquors and that it be studied collaboratively, with respect to its application to cordials and liqueurs, before adoption as final action.

(3) That the official modified Marsh test and the official Milos test, 16.39 and 16.41 for caramel, be deleted, final action.

(4) That the study of colorimetric methods for fusel oil be continued.

(5) That the study of the method 16.22–16.23 for fusel oil be continued.

(6) That the official method, 16.29, for methanol in distilled liquors by the immersion refractometer method be studied in the light of the findings of Beyer and Reeves, *This Journal*, 28, 800 (1945).

(7) That an Associate Referee be appointed to determine if the immersion refractometer method for alcohol in 15.4(c) should be incorporated in Chapters 14 and 16.

(8) That the rapid method for proof of distilled spirits, as recommended in the report of the Associate Referee on obscuration test for proof of distilled spirits be adopted as official, first action.

(9) That study be continued of the official Denigès method for methanol, 16.25, and the tentative method for methanol in 39.161 and 39.162 to bring about uniformity in these procedures so far as possible.

Cordials and Liqueurs:

It is recommended—

(1) That section “16.45 Specific Gravity, see 14.3” be changed to read “16.45 Specific Gravity, proceed as under 16.2.”

(2) That a collaborative study be made of methods for caramel in cordials and liqueurs.

(3) That a collaborative study be made of the two tentative methods for total solids, i.e., 16.51(a) From sp. gr. of dealcoholized sample, and 16.51(b) By evaporation.

(4) That a collaborative study be made of the tentative method for total acidity, 16.62.

CACAO PRODUCTS

It is recommended—

(1) That the method for lecithin in cacao products be further studied.

(2) That the method for lactose in cacao products, reported this year, be studied to increase the rapidity of the method and to correct for effect of the presence of higher sugars in corn sirup solids, and that collaborative work be conducted.

(3) That the study of methods for maltose and cacao ingredient be continued.

(4) That the tentative method for pectic acid, 19.16, be revised as recommended by the Referee.

(5) That the method for pectic acid in milk chocolate be further studied.

(6) That the method of separation of fat, 19.25, when used on milk chocolate, be studied and compared with the method for refractory sample proposed by Ferris, *This Journal*, 31, 728 (1948).

CEREAL FOODS

It is recommended—

(1) That procedures adopted as official, first action, last year, *This Journal*, 31, 79, for the determination of phosphorus in cereals and cereal products be adopted as official, final action, and the study be discontinued.

(2) That the studies on determination of starch in raw and cooked cereals be continued.

(3) That the tentative method for the determination of fat acidity in grain and flour (20.18–20.21, incl., and 20.76) be adopted as official, first action, and the study continued.

(4) That a study of the application of the method for reducing and non-reducing sugar in flour, 20.28–20.30, incl., be made to the determination of sugars in bread and other bakery products, with special consideration given to the article on this subject published by R. M. Sandstedt and G. C. Fleming, *This Journal*, 30, 550–552.

(5) That the tentative method for benzoic acid in flour (20.53) be replaced by the method proposed by the Associate Referee for wheat flour and adopted as official, first action, as a qualitative test and the study continued.

(6) That work on methods for determination of available CO₂ in self-rising flour, containing added CaCO₃, be discontinued.

(7) That the methods for the determination of lactose in bread be further studied.

(8) That the method for the determination of fat and fat number in bread as proposed by the Associate Referee in this year's report, replace 20.86 and be adopted as official, first action.

(9) That the method proposed by the Associate Referee in this year's report for the determination of proteolytic activity of flour and malted wheat flour be adopted as official, first action, and that the work be continued.

(10) That the methods for soybean flour, *This Journal*, 31, 81 (1948) for moisture, deleting "20.2," ash, nitrogen, and oil or petroleum benzene

extract, be adopted as official, first action, and the study be continued.

(11) That the study on the detection and determination of soybean flour in cereal products be discontinued.

(12) That the method proposed by the Associate Referee in this year's report for the determination of added inorganic material in phosphated flour be adopted as official, first action, and the study continued with elimination of self-rising flour.

(13) That the method referred to in *This Journal*, 25, 83-84, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicability to bakery products containing eggs.

(14) That the study of methods for the determination of albumen in noodles and macaroni products be conducted.

(15) That the study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(16) That the study on the determination of moisture in flour products containing sodium bicarbonate as one of its constituents be continued.

(17) That the study on the determination of bromates in flour be continued.

(18) That the tentative methods 20.32 to 20.38, incl., and 20.60, be dropped.

BAKING POWDERS

It is recommended—

(1) That the tentative, modified McGill method, 17.21, be dropped.

(2) That the tentative qualitative test for phosphoric acid, 17.31, be adopted as official, first action.

(3) That the tentative methods and the oven modification No. 1 as set forth in the Associate Referee report for this year on residual CO_2 in baking powder be adopted as official, first action.

(4) That the HCl (1+2), be adopted as alternate for H_2SO_4 (1+5) in the official method for total CO_2 , 17.4 and 17.6.

(5) That the available CO_2 , 17.9, be determined by subtraction of residual CO_2 (see recommendation 3) from the total CO_2 , 17.6.

(6) That the official gasometric method 17.8 be dropped, final action.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That the collaborative study of the reflux method for determination of peel oil in citrus fruit juices and the use of the modified oil separation trap be continued.

(2) That collaborative work be continued on the method for determination of beta-ionone where small amounts are present.

(3) That collaborative studies on the Ripper method for determination of aldehydes in spirits as applied to lemon oils and extracts be continued.

(4) That collaborative studies of the methods proposed by the Referee for determination of esters in lemon extract be continued.

(5) That collaborative studies on the Seeker-Kirby method for determination of esters in lemon and orange oils (Dept. of Agri. bull. 241) be continued.

(6) That collaborative studies of extract methods containing both isopropyl alcohol and acetone be continued.

(7) That collaborative study of the photometric method for determination of vanillin and coumarin be continued.

(8) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts with special reference to the automatic extraction of vanilla and coumarin.

(9) That the study of emulsion flavors be continued.

(10) That studies on maple concentrates and imitations be continued.

(11) That study of the method for determination of diacetyl, published in *This Journal*, 25, 255, be continued.

(12) That the Referee study collaboratively the modification of 25.23, in lemon, orange, and lime extracts, as given in last year's report, *This Journal*, 31, 202 (1948).

(13) That the Referee study collaboratively the modifications of 25.54 for alcohol in almond extract as given in last year's report, *This Journal*, 31, 203 (1948).

(14) That the methods for vanilla resins in vanilla extract, 25.15 and 25.16, be studied collaboratively.

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That study of methods for determining fruit and sugar content of frozen fruits be continued.

(2) That further collaborative study be made of the method for the electrometric titration of acidity.

(3) That further study be made of methods for separating and determining fruit acids.

(4) That procedures as given by the Associate Referee in his 1948 report for the rapid determination of water-insoluble solids and for the determination of seeds of berry fruits be adopted as tentative and that the procedures be subjected to collaborative study.

(5) That 26.18(a) be changed as recommended by the Referee (first recommendation).

(6) That the sentence beginning "If recoveries are low . . ." in 26.19, Note (3), be deleted.

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the method for the determination of shellac on confectionery *This Journal*, 31, 196 (1948) modified as described in this year's report of the Associate Referee, be adopted as official, first action, and that further collaborative work be conducted on it.

(2) That the method of the Java Sugar Experiment Station for unfermented reducing substances in molasses, adopted as a tentative method last year, be made official, first action, with the changes recommended by the Referee.

(3) That the study for the determination of moisture be continued.

(4) That study be continued on tables of density of solutions of sugar at various temperatures.

(5) That the Zerban and Martin values for refractive indices of dextrose and invert sugar solutions (*This Journal*, 27, 295) be subjected to collaborative study.

(6) That the official method for the determination of free acid in honey, 34.99, be further studied collaboratively with a view to establishing the end point more accurately.

(7) That study be made of methods for the detection of adulterants in honey, particularly commercial sirups.

(8) That study be continued on the determination of dextrose, maltose, and dextrans, by copper reduction methods in carbohydrate mixtures.

(9) That the tentative methods, 34.133–34.155, inclusive, be subjected to collaborative study.

(10) That the procedures in N.B.S. Circular C440, pp. 324–334, for measurement of transmittancy of solutions of commercial sugar products, be study collaboratively.

(11) That the Somogyi Modification (*J. Biol. Chem.*, V. 160, p. 61, 1945) of the tentative micro method for reducing sugars (34.63) be studied.

(12) That Ofner's method for the determination of invert sugar in the presence of sucrose be made official, first action.

(13) That the method 34.8 for solids by refractometer be amended by adding: "In liquid products containing invert sugar, correct the per cent solids obtained from 44.7 by adding 0.022 for each per cent invert sugar present in the sample." (First recommendation.)

REPORT BY REPRESENTATIVES OF THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL

By W. H. MacINTIRE

This report is being presented in behalf of our beloved and lamented confrere, Dr. H. J. Patterson, of whom the Chairman of the Board of Governors of the Institute wrote so recently—

"I was tremendously sorry to learn of the death of Dr. Patterson. He was a great character and a man of profound accomplishment."

As though there he had a premonition of his demise, our beloved Past

President had suggested that he be succeeded in his assignment as representative from this Association. Because of his sustained interest in the objectives of the Institute, however, he acceded to the urgent request that he continue to serve under designation of this Association. Through his advice, counsel, and interest Dr. Patterson contributed much to the success of this Institute.

The activities of the Institute during the past year have covered a wide range of research. The program included exploratory studies of new chemical compounds as insecticides and fungicides; the influence of inert extenders on the behavior of insecticidal dusts; development of formulae for aerosols; exploration of mildew-proofing agents; studies on moth proofers; research in chemotherapy and in analytical procedures for organic phosphates.

The Institute has continued to function upon basis of cooperation and collaboration between the research chemists and the biological experimenters of the academic institutions and those workers of like designation among the industrial organizations. Such joint efforts have led to the discovery of new and better materials for plant protection and have pointed the way to profitable manufacture and effective use of the various new compounds. During the past year guidance and direction by the Institute have been sought by 19 collaborating agencies, laboratories, and corporations.

The policies and functions of the Institute during the past year were directed by its Board, which was comprised of Dr. W. C. O'Kane (Chairman) of the New Hampshire Experiment Station, Dr. D. M. DeLong, Ohio State University, and A. M. Boyce of the Riverside unit of the California Experiment Station, as representatives of Entomology; Dr. H. W. Thurston (Vice-Chairman) of the Pennsylvania Experiment Station, Dr. J. G. Horsfall, Director of the Connecticut Experiment Station, and Director C. R. Orton of the West Virginia Experiment Station representing Plant Pathology; Dr. S. E. A. McCallan of Boyce Thompson Institute for National Research Council, and H. J. Patterson and W. H. MacIntire as designates of the Association of Official Agricultural Chemists.

The Institute continues to serve as a liaison between the research worker and the user of the worker's findings. Its usefulness has been recognized by those industrial agencies that need factual information and scientific guidance. To be on its Board has been an educational and inspirational experience to your remaining representative.

Approved.

REPORT OF THE SECRETARY-TREASURER

By HENRY A. LEPPER

The meeting of the executive committee was held Sunday, October 10, 1948, at 10 A.M., in the Board Room of the Cosmos Club. All members of the committee were in attendance. The audit of the firm of Snyder, Farr, and Company was presented and accepted.

ASSOCIATION OF OFFICIAL AGRICULTURAL
CHEMISTS, INC.

BALANCE SHEET—SEPTEMBER 30, 1948

ASSETS

Current Assets:

Cash, Lincoln National Bank.....	\$20,964.29	
Office cash fund.....	56.14	\$21,020.43

Accounts receivable.....	\$ 4,632.46	
Less reserve for doubtful accounts.....	163.90	4,468.56

Accrued interest receivable, Government bonds.....		250.00
Inventories.....		6,161.37

<i>Total Current Assets.....</i>		<i>\$31,900.36</i>
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Fixed Assets:

Furniture and fixtures.....		756.01
<i>Investments.....</i>		<i>42,121.00</i>

<i>Total Assets.....</i>		<i>\$74,777.37</i>
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SURPLUS

<i>Balance, October 1, 1947.....</i>		<i>\$72,408.75</i>
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Add: Net profit for the fiscal year ended September 30, 1948.....	\$ 2,379.92	
Recreation fund transferred from old account ..	39.00	
		2,418.92

		\$74,827.67
Less: Old returned checks written off.....		50.30

<i>Balance, September 30, 1948.....</i>		<i>\$74,777.37</i>
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It has been our custom through the years in evaluating the financial status of our *Journal* to balance the income from subscriptions and advertising against printing and mailing costs. On this basis we have considered *The Journal* to be self-sustaining. This year we had the auditor estimate the cost of publishing *The Journal* by allocating administrative expenses to gross profits on sales of *Methods of Analysis* and *The Journal* based on the ratio of sales of each to total sales. Such expense is properly chargeable to *The Journal*, and with this addition we find that it has cost

the Association \$1,851.36 from our general operating fund to publish and distribute this year's volume of *The Journal*. Your executive committee recognizes that scientific journals generally do not balance their budgets by depending solely on subscription returns. The committee further regards our *Journal* as a contribution to scientific literature and supplemental to our publication of the *Methods of Analysis*. To serve such interests it is therefore recommended that the Association contribute from its general fund the support necessary for *The Journal* in lieu of an increase in the subscription price with the possible decrease in the number of subscribers. It further suggests the appointment of a subcommittee of three of its members to consider and put into effect steps which might be taken looking toward an increase in the number of subscribers. To expedite the work, members located in Washington should constitute the subcommittee, and Messrs. Queen, White, and Lepper are suggested.

The association has received word from G. E. Gratton of the Department of agriculture of Canada that through a change of position he will no longer be able to attend our meetings and continue his activities in the Association. The vacancy thus created on Subcommittee A has been filled by the appointment of John B. Smith of Rhode Island. The terms of C. S. Ferguson on Committee D and H. J. Fisher on Committee B expire this year and their reappointment is recommended. The present chairman of the Committee, W. F. Reindollar, was reappointed.

During the past year the addressing machine authorized for purchase at the last meeting and an adding machine authorized during the year by the executive committee were purchased at a total cost of \$475.01. These have expedited the clerical and routine work of the business office in complete fulfillment of our expectations. The committee also authorized the purchase of two additional projectors and screens costing \$261.00, and all in attendance at this meeting, when they are available for the first time, appreciate their worth in the presentation of reports at the several sectional meetings. Their usefulness should increase as the members now know of their availability.

Approved.

REPORT OF COMMITTEE ON NECROLOGY

JOSEPH A. AMBLER

Dr. Ambler was a native of Danbury, Connecticut, born June 30, 1889; he died October 6, 1948, in New Orleans, La. He was chief organic chemist with the Department of Agriculture Research Division in New Orleans. Dr. Ambler received his doctor's degree in organic chemistry from Yale University in 1913. He joined the Department of Agriculture as a chemist in 1917. In 1937 he was cited by the department for explaining the effects of contaminants on the production of sugar.

HORACE T. HERRICK

Dr. Herrick was born April 22, 1887, a native of Brooklyn, N. Y. and died October 7, 1948, in Washington, D. C. He was graduated from Lawrenceville School and Princeton University and received a degree in chemical engineering from Columbia University. One of his outstanding accomplishments in 22 years with the U. S. Department of Agriculture was his assistance in setting up four regional research laboratories to search for new industrial outlets and markets for farm crops. Prior to his appointment in 1946 as Special Assistant to the Chief of the Bureau of Agricultural and Industrial Chemistry, he was successively head of the Bureau's Color and Farm Waste Division; head of the Industrial Farm Products Research Division; and special assistant on the four regional research laboratories project.

WILLIAM F. HAND

Dr. Hand was born December 1, 1873, in Shubuta, Mississippi, and died September 25, 1948. Teacher and scientist for 53 years, and vice president of Mississippi State College for 13 years, Dr. Hand designed and built the Mississippi State Chemical Laboratory which is dedicated to him. He was graduated from Mississippi Agricultural and Mechanical College in 1895, and from Columbia University in 1903, with the degree of Ph.D. He was President of the Association in 1921 and President of the American Society of Feed Control Officials. In recent years he prepared all regulatory feed, fertilizer, paint, food, drug, and petroleum laws for the State of Mississippi.

HARRY J. PATTERSON

Dr. Patterson was born in Yellow Springs, Pa., December 17, 1866, and died on September 11, 1948, in Boston, Mass. He was dean emeritus of the University of Maryland, College of Agriculture and Experiment Station. Before coming to the University of Maryland, Dr. Patterson held positions at Pennsylvania State College. He was Secretary of the Maryland State Board of Agriculture from 1908 to 1916. His work was in the field of chemistry, feeding and fertilizing problems; methods of rendering phosphates available to crops, etc.

ARTHUR E. PAUL

Dr. Paul was born in Germania, Pa., September 29, 1874, and died on January 1, 1948. He was originally appointed as an assistant chemist at the Chicago Station of the Food and Drug Administration in 1909. He served successively as Chief Chemist of the Chicago Station, Chief of the Cincinnati Station, Chief of the Chicago Station, and Assistant Chief of the Central District. Dr. Paul was particularly interested in the scientific aspects of food and drug work. He served faithfully in the work of the

Association of Official Agricultural Chemists and was president of that body in 1932.

ALFRED E. TAYLOR

Dr. Taylor was born in Portland, Connecticut, October 16, 1871, and died in Washington, D. C., on May 30, 1948. Dr. Taylor reported to the old Bureau of Chemistry in April, 1907, following seven years as a chemist in the Customs Service. From 1918 until his retirement in October, 1941, he supervised and coordinated the work on imports in the Food and Drug Administration. Dr. Taylor was graduated from Harvard University in 1894 with an A.M. Degree, and from Cornell University in 1896, with a Ph.D. degree. At the time of his retirement in 1941, Dr. Taylor had served the Federal government for 41 years.

E. PECK GREENE

Mr. Greene was born in Clarksville, Arkansas, July 15, 1884, and died at Tallahassee, Florida, April 4, 1948. He was educated in the public schools of Florida and at the University of Florida at Lake City. In 1909 he joined the State Department of Agriculture and at the time of his death was one of the oldest state employees in point of service, having served for more than 35 years. Mr. Greene was greatly interested in plants, shrubs, and flowers, and the E. Peck Greene Park in Tallahassee, Florida, has been named by the city in his honor.

ARTHUR L. SULLIVAN

Mr. Sullivan was born in Suncook, New Hampshire, in 1881, and died August 16, 1948, in Baltimore, Md. He was graduated from the University of New Hampshire in 1902, with the degree of B.S. He worked as an assistant chemist with the U. S. Bureau of Internal Revenue from 1903 to 1907, when he transferred to the U. S. Bureau of Chemistry as a food and drug inspection chemist. In 1912 he was made Chief of the Boston Station of the Food and Drug Administration. In 1915 he returned to Washington to be in charge of the Washington laboratory. He was made Chief of the Baltimore Station in 1917, serving until 1920, when he was made Food and Drug Commissioner for the State of Maryland. As a State food official Mr. Sullivan cooperated in the enforcement of the Federal law. The improvement of the sea-food industry in Maryland is due in no small part to his interest in that industry and his enforcement of laws, especially those covering sanitation.

J. W. SALE, *Chairman*
W. L. HILL
JOHN B. SMITH

REPORT OF THE COMMITTEE ON NOMINATIONS

Your committee presents the following designations and recommends their election to the offices indicated:

President: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

Vice-President: W. A. Queen, Food & Drug Administration, Washington 25, D. C.

Secretary-Treasurer Emeritus: W. W. Skinner, Kensington, Md.

Secretary-Treasurer: Henry A. Lepper, Food & Drug Administration, Washington 25, D. C.

Additional Members of the Executive Committee: H. A. Halvorson, State Chemist, St. Paul, Minn.; W. B. White, Washington, D. C.; H. J. Fisher, Conn. Agricultural Experiment Station, New Haven, Conn.; G. H. Marsh, Dir. Chemistry Division, Montgomery, Ala.

Approved.

W. H. MacINTIRE, *Chairman*

J. J. T. GRAHAM

K. L. MILSTEAD

REPORT OF COMMITTEE ON RESOLUTIONS

Acknowledging our indebtedness to the officers and members of the Association who were responsible for making this annual meeting a useful and profitable gathering, we hereby extend to all and to each of them our sincere thanks for their able performance of duties and our congratulations upon the success of this 62nd annual meeting; and

Whereas, the management and employees of the Shoreham Hotel have afforded us a most convenient and delightful meeting place and most courteous treatment, therefore be it

Resolved, that we extend to the hotel our thanks and this expression of appreciation of their effective aid in making our stay pleasant in all respects; and

Whereas, we recognize the value of publicity to the best functioning of the Association, therefore, be it

Resolved, that we extend our thanks hereby to the press and their representatives who have covered this meeting; and

Whereas, we all acknowledge our debt of gratitude of the Referees and Associate Referees, and those who have collaborated with them in carrying on during the past year the real work of the Association, therefore be it

Resolved, that we by this expression assure them of our real appreciation of their efforts and of our earnest intention to assist them in the future.

Approved.

H. J. WICHMANN, *Chairman*

J. L. ST. JOHN

GUY G. FRARY

CHANGES IN OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE SIXTY-SECOND ANNUAL MEETING, OCTOBER 11, 12, AND 13, 1948*

The changes in the methods of the Association recorded below become effective March 17, 1949, the thirtieth day after the date of publication of this Report, Feb. 15, 1949, as provided in section 5 of the by-laws as amended at the 1948 convention (see page 38).

The classification of methods employed in this report follows that of previous years. The classification provided by the by-laws as amended this year (see p. 33) will be applied to revisions made at the 1949 meeting for publication in the contemplated seventh revision of *Methods of Analysis*, in 1950.

1. SOILS

No additions, deletions, or other changes.

2. FERTILIZERS

(1) The following was adopted as official, first action.

NITROGEN IN AMMONIUM NITRATE

Formaldehyde Titration Method

(May also be adapted to Ammonium Sulfate)

Weigh out 7.004 or 14.008 g of sample and make up to 250 or 500 ml. Pull off 25 or 50 ml and put into a 300–500 ml Erlenmeyer (ca. 1.5 g may be rapidly weighed and washed directly into flask). Add ca. 1 ml of reagent formaldehyde for each 0.1 g of sample in aliquot. Make total volume 150–200 ml and allow 5 min. before titrating with 0.25–0.50 *N* sodium hydroxide, using 5 drops of phenolphthalein as indicator. Titrate until there is no perceptible color change at the point of contact, or until the proper color of pink persists. Run a blank on the formaldehyde.

$$\% \text{ Nitrogen} = \frac{\text{Net ml of NaOH} \times \text{Normality} \times 2.8016}{\text{Wt. of Sample}}.$$

(2) The official, first action, changes reported in *This Journal*, 31, 71, under fertilizers (2) (a), (b), (c), (d), (e), and (f) were made official, final action.

(3) The title "Acidulated samples" of 2.16(a) first line was changed to "Acidulated samples and mixed fertilizers," and lines 1 to 11 beginning with "after" and ending with "retentiveness" were replaced with the following, official, first action.

"After removing water-soluble P_2O_5 , 2.13, transfer the filter and residue, within a period not to exceed 1 hour, to 200 or 250 ml flask containing 100 ml NH_4 citrate soln previously heated to 65°. Close flask tightly with a smooth rubber stopper, shake vigorously until filter paper is reduced to pulp, relieve pressure by momentarily removing stopper, and proceed by one of the following methods: (1) Loosely stopper flask to prevent evaporation, place in water bath regulated to maintain contents of flask at exactly 65°, keep level of H_2O in bath above that of citrate soln in flask, and

* Unless otherwise given all references in this Report are to *Methods of Analysis*, A.O.A.C., 1945.

shake every 5 min; (2) continuously agitate contents of stoppered flask by means of apparatus equipped to maintain contents of flask at exactly 65°. At expiration of exactly 1 hour from time filter and residue were introduced, remove flask from bath or apparatus and immediately filter contents as rapidly as possible thru Whatman filter paper No. 5 or other paper of equal speed and retentiveness."

(4) The words "other than basic slag" 2.16(b), first line, were deleted, first action.

(5) That part of 2.17, line 3, beginning "in acidulated samples" to the end of the section, was deleted, first action.

(6) Methods 2.18, 2.19, and 2.20 for citric acid-soluble phosphoric acid in basic slag were deleted, first action.

(7) The following methods were adopted as official, first action.

FREE WATER IN FERTILIZERS

Air-flow Method

APPARATUS

(A) *Manifold Assembly*.—A metal box $10\frac{1}{2} \times 2\frac{1}{2} \times 1\frac{1}{2}$ inches in size† is equipped with a $\frac{1}{2}$ -inch nipple centrally located on one side for attachment to a vacuum line, and 6 $1\frac{1}{2}$ -inch tapered stopper seats evenly spaced along the top to accommodate No. 6, one-hole, rubber stoppers. A $1\frac{1}{2}$ -inch length of light metal tubing, $\frac{1}{2}$ inch in diameter, extends thru each rubber stopper to a height of $\frac{1}{2}$ inch above the surface for the purpose of centering a fritted glass crucible over the hole in the stopper. Since the crucible is held in place by suction, it is necessary to grind a smooth surface on the lower edge of each fritted glass crucible and on the surface of the stopper in order to insure an air-tight connection between the edge of the crucible and the stopper when air is being drawn thru the sample in the crucible.

(B) *Crucibles*.—Pyrex glass, approximately $1\frac{1}{2}$ inches tall, $1\frac{1}{8}$ inches in diameter and having a $\frac{1}{8}$ -inch fine-porosity fritted glass plate. Individual crucibles of a set should all have approximately the same porosity. A matched set may be obtained by selecting several that pass a given quantity of air at constant pressure in approximately the same length of time.

(C) *Vacuum Gage*.—A standard instrument for insertion in the rubber vacuum line between the source of suction and the manifold.

(D) *Constant Temperature Oven*.—A standard laboratory oven, preferably of the type vented so that incoming air passes directly over the heating coils.

DETERMINATION

Weigh 2 g of prepared sample, 2.2, in a tared, fritted glass crucible. (Extremely hygroscopic or damp materials should be weighed out of difference in covered crucibles.) Place crucible on manifold in the oven at 60°. Aspirate for 2 hours under 15 in. of vacuum. Cool in desiccator, for 30 min. and reweigh. Calculate percentage loss in weight.

Vacuum-drying Method

DETERMINATION

Place 4 g of prepared sample, 2.2, in a short-type, tared weighing dish of a size not less than 2 in. in diameter. (Extremely hygroscopic or damp materials should

† An illustration of this apparatus is given in *This Journal*, 31, p. 235 (1948).

be weighed out of difference in covered dishes.) Place in a vacuum desiccator over anhydrous magnesium perchlorate for 16 hours under not less than 25 in. of vacuum. Reweigh and calculate percentage loss in weight.

NOTE: A drying period of 16 hrs. represents over-night drying. The average type of fertilizer will release its free moisture in 3-6 hrs. However, over-night drying insures best results, especially on very damp materials and on samples containing high amounts of adsorbed water.

Vacuum-Desiccation Method

Place 2 grams of sample in a tared low-form weighing dish (4 grams of sample may be used with large weighing dishes, 1.5-2 inches in diameter) and place it in a vacuum desiccator at 25-30°C. over anhydrous magnesium perchlorate (or equivalent desiccant) for 16-18 hours under not less than 20 inches of vacuum. Report percentage loss in weight as moisture.

(8) The tentative method for moisture by distillation with toluene, 2.5 and 2.6, was deleted.

(9) The parenthetical statement in the method for moisture, 2.4 "(Not applicable to samples containing compounds other than H_2O that are volatilized at the temp. of drying)" was changed to "(Not applicable to samples that yield volatile substances other than H_2O at the temp. of drying)."

(10) The wording of the directions in the official method for moisture—by drying, was changed to read "Heat 2 g of prepared sample, 2.2, for 5 hours in oven at temp. of 99-101°. In case of $NaNO_3$, $(NH_4)_2SO_4$ and potash salts heat to constant weight in an oven at temp. of 129-131°"; and the heading "Moisture" was changed to "Water."

3. AGRICULTURAL LIMING MATERIALS

(1) The method for neutralization value of calcium silicate slags 3.11(a), (p. 44) was annotated by the statement "without correction for sulfide content."

(2) The following method was adopted as tentative.

SULFIDE SULFUR IN CALCIUM SILICATE SLAGS

REAGENTS

(a) *HCl* (1+4).

(b) *Zinc dust*.—C. P. grade, of low lead content.

(c) *Absorbent*.—Dissolve 20 g of $CdSO_4 \cdot 2\frac{1}{2} H_2O$ in H_2O and make to 1 liter. Adjust reaction to pH 5.6 by means of either potentiometric titration or colorimetric comparisons on a separate 50-ml aliquot and matching a buffer of same pH.

(d) *Standard alkali*.—Prepare 0.1 N NaOH from CO_2 -free NaOH solution and use of CO_2 -free H_2O .

(e) *Standard acid*.—0.1 N HCl.

APPARATUS

A 250-ml Erlenmeyer evolution flask to fit No. 5.5 stopper, a 60-ml separatory funnel fitted into the stopper and the lower third of its stem drawn to a 2 mm open-

ing and bent upwards at its extremity. The funnel in the stopper is adjusted so that its stem is $\frac{1}{2}$ inch from the bottom of the evolution flask. Two 25 × 150 mm test tubes are fitted with No. 4 two-hole stoppers and inlet and outlet of 6 mm tubing, which serve to contain jointly the 50 ml of absorbent. The tubes are in series and should be placed in a 600-ml beaker filled with cold water, and the beaker placed upon a tripod on level with the evolution flask. Another test tube of similar size is half-filled with H₂O and clamped in position between evolution flask and absorbent solution to provide trap for HCl vapor.

DETERMINATION

Weigh 1 g of minus 80-mesh slag into the evolution flask; add 1 g of Zn dust and wash down sides with 5–10 ml H₂O; mix contents by means of flattened end of stirring rod and connect the flask. Introduce 50 ml of HCl (1+4) into separatory funnel; open stopcock to allow acid to flow into reaction flask, swirling the contents meanwhile. If necessary, apply pressure to effect complete transfer of acid from funnel. Close stopcock and continue the swirling 5 min. at brief intervals. Apply heat; continue the swirling until boiling starts and then regulate flame to maintain active boiling, altho not too vigorous, thruout 10 min. Keep the contents of the HCl vapor-trap near the boiling point thruout dissolution of the slag, then snap off the rubber tubing at the CdSO₄ intake quickly, taking care not to allow a backing-up of the contents. Cut off heat, discard contents of the evolution flask and of the scrubber tube, and set up apparatus for the next determination.

Filter the CdS suspension on a 9 cm gravity filter leading into a 250 ml Erlenmeyer flask and wash with H₂O to an overall volume of 100 ml. Add 4 drops of a 0.2 per cent soln of methyl red and agitate vigorously while titrating slowly to the exact orange-yellow tint of the reference soln. That soln comprises 50 ml of the CdS soln diluted to 100 ml, with identical concentration of indicator and contained in a 250-ml Erlenmeyer flask. Should the end point be passed with resultant precipitation of Cd(OH)₂, introduce 1–2 ml of 0.1 N HCl, and allow to stand until the precipitate disappears and then complete the titration dropwise under vigorous agitation. Divide by 2 the ml of net 0.1 N NaOH used to obtain the per cent CaCO₃-equivalence of the sulfide sulfur in the sample. The ml of 0.1 N NaOH × .0016 = g sulfide sulfur per determination; and the latter, times 100 = percentage of sulfide S.

4. COSMETICS

- (1) The following method was adopted as official, first action.

PYROGALLOL IN HAIR DYES

Qualitative test

Add 5–10 ml of sample to separatory funnel containing ca 0.5 g of NaHSO₃ and extract with 2 or 3 successive 30-ml volumes of ether. Filter ether extracts thru cotton and evaporate to dryness on steam bath. Dry in oven at 100°C for 30–60 min. Pulverize residue, mix well, and take melting point. If it does not melt between 131° and 134°C, sublime and again take melting point, which should fall within above range. Mix small portion of residue with equal quantity of sublimed pyrogallol and determine the melting point; it should not change.

Quantitative Determination

REAGENTS

Ferrous Tartrate Reagent.—Dissolve 1.00 g of sodium potassium tartrate (Rochelle salt) and 0.200 g of FeDO₄ · 7 H₂O in water and dilute to 100 ml in a volumetric flask. PREPARE FRESH DAILY.

Sodium Acetate Solutions.—Dissolve 15.00 g $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ in water, bring to room temp. and dilute to 100 ml.

Aluminum Cream—34.19(b).

Standardization.—To six 100 ml volumetric flasks add from buret 2.50, 5.00, 7.50, 10.00, 12.50, and 15.00 ml of standard pyrogallol solution (Reagent Grade, 0.2000 g/500 ml). Develop color as follows on not more than three standards at a time, and make readings within 15 min. after color is developed. Pipet into flasks 10 ml each of Na acetate soln and ferrous tartrate reagent, dilute to volume and mix. Using 1 cm or $\frac{1}{2}$ inch cells, measure optical density of solutions with photometer or spectrophotometer at 540 μ . With neutral wedge filter photometer, filter designated No. 56 (5.8 mm of Corning didymium #512, 2.0 mm of Jena VG 3, 2.0 mm of Jena BG 18, and 4.5 mm of Corning yellow shade yellow #351) is more suitable than filter No. 54. With filter photometers obtain zero point by reading "blank" soln containing 10 ml of Na acetate and ferrous tartrate reagents in 100 ml. Draw standard curve, plotting concentration of pyrogallol against photometer readings, on large-scale graph paper so that pyrogallol can be read to 0.01 mg. A straight line should be obtained between concentrations of 1 mg and 6 mg per 100 ml. With spectrophotometer use freshly prepared "blank" as reference soln. Draw standard curve as directed above, or, if straight line passing thru origin is obtained, the average value of k may be calculated from the formula

$$k \frac{D}{c}$$

where

k is extinction coefficient

D is measured optical density of solution

c is concentration of pyrogallol in mg per 100 ml

This value of k may be used to calculate the concentration of unknowns directly from optical density readings.

Liquid Dyes

Extract a convenient aliquot of sample (usually 10 ml are sufficient) by one of the following methods. In handling sample give it a minimum of exposure to air, as pyrogallol is readily oxidized.

1. **Continuous Extraction.**—Pipet sample aliquot into suitable continuous extractor containing ca 0.3 g of NaHSO_4 . Extract with ether until pyrogallol is completely removed (3–7 hours, depending upon efficiency of extractor). Determine time required for each extractor under a certain set of conditions by extracting an aqueous soln of known pyrogallol content or by testing for complete extraction as follows: After the extraction is thought to be complete, remove flask containing ether and replace it with one containing fresh volume of ether and continue extraction for 30–60 min. Treat this extract as directed below, and use 50 ml aliquot of filtrate to develop color. Evaporate ether extract on steam bath to volume of 8–10 ml and continue evaporation at temp. not exceeding 40° until odor of ether is completely gone. Dissolve residue in 20 ml of water and wash completely in 100 ml volumetric flask. Dilute to volume and mix. (If the liquid sample contained chlorophyll, treat residue from ether extraction as described for residue obtained from evaporation of ethyl acetate extract in method for henna powder dyes, beginning, "Add ca 10 ml water to beaker and loosen the residue. . ."). Filter thru dry paper and discard first 20 ml of filtrate. (If determination cannot be completed same day extraction is made, let ether extract stand overnight, preferably in refrigerator, before ether is evaporated. Do not let aqueous soln stand overnight.) Use suitable aliquots of filtrate to develop color as directed under "Standardization," beginning "Pipet

into flasks. . . .” If 5 ml aliquot contains more than 6 mg of pyrogallol, make suitable dilution in volumetric flask and use aliquots of diluted soln to develop color. For final calculation use average of results obtained on at least two aliquots of different sizes, preferably containing between 2 mg and 5.5 mg of pyrogallol. Calculate to g/100 ml in original sample.

2. *Extraction in Separatory Funnels.*—Pipet sample into 125 ml separatory funnel containing ca 0.3 g of NaHSO_3 and extract 6 times with ether. For each extraction use volume of ether equal to 3 or 4 times volume of sample and shake vigorously for one min. Filter ether extracts successively thru cotton wet with ether. (Six extractions carefully made will completely remove the pyrogallol. If desired, a 7th may be made and used to test for complete extraction as described under “Continuous Extraction.”) Evaporate the combined ether extracts as directed under “Continuous Extraction.”

Henna Powder Mixture

Weigh 0.9 to 1.1 of thoroughly mixed sample in paper extraction thimble. Cover sample with small piece of cotton and place thimble in Soxhlet extractor. If temperature and humidity conditions are such that water will condense on condenser, connect tube containing drying agent to outlet of condenser. Extract 5 hours with ethyl acetate having minimum purity of 99% (N.F. VIII or better quality). Boil at such rate that solvent siphons off 15 to 20 times per hour. If ethyl acetate extract is clear, evaporate to dryness as directed below. If extract contains any sediment, evaporate to ca 75 ml if necessary, cool to room temperature, and completely transfer to 110 ml glass-stoppered volumetric flask. Dilute to volume and mix. Filter thru dry paper, taking precautions to prevent evaporation of solvent. Pipet 100 ml of filtrate into 250 ml beaker and evaporate to volume of ca 5 ml on hot plate or steam bath. Continue evaporation to complete dryness at temperature not exceeding 40°. Add 10 ml water and loosen residue with stirring rod. Pour into 50 ml volumetric flask. Rinse beaker 4 or 5 times with small volumes of water and add rinsings to flask. Add 1.2 ml alumina cream, dilute to volume, mix and filter thru dry paper. Extract 25 ml by one of methods given under “Liquid Dyes.” (If extraction cannot be started immediately add ca 0.4 g of NaHSO_3 to filtrate and hold no longer than overnight.) Calculate to per cent pyrogallol in original sample.

(2) The following methods were adopted as official, first action.

CONSTITUENTS OF FACE POWDERS

Fats and Fatty Acids as Stearic Acid

Weigh about 2 g of the powder into glass-stoppered 250-ml Erlenmeyer flask. Add 30 ml of benzene and swirl to mix thoroly. Add 10 ml of HCl , and swirl, removing stopper frequently to allow escape of CO_2 from carbonates. When pressure has spent itself, add 50 ml of petroleum ether, and shake cautiously with periodic removal of stopper until pressure again subsides. Then shake vigorously about 50 times. Decant ether layer thru a pledget of cotton into flask containing a few glass beads, that has been weighed with a similar flask as a counterpoise. (This decantation involves no danger of loss, for the particles of powder are tenaciously retained in the acid layer.) Again add 50 ml of petroleum ether and repeat shaking and decantation. Repeat with a third 50-ml portion of petroleum ether. Evaporate to dryness on steam bath under hood. Place in a draft oven at 100 degrees C. for one hour, heating flask used as a counterpoise at the same time. Remove flasks, cool, and weigh as stearic acid.

Total Zinc

REAGENTS

(a) *Wulfig's precipitant*.—Dissolve 80 g of finely ground ammonium carbonate in a mixture of 90 ml of NH_4OH and 375 ml of H_2O , and add 475 ml of 95% ethyl alcohol, which may or may not cause precipitation, depending on the temperature. Let any precipitate settle, and use supernatant soln.

(b) *Wash soln*.—Mix equal volumes of Wulfig's precipitant and 95% ethyl alcohol.

DETERMINATION

Weigh ca 2 g of the powder into a platinum dish and ignite to light gray ash at 600–650°C. Do not heat longer than necessary. With the aid of a wide-mouth funnel, transfer ash to a 500-ml glass-stoppered Erlenmeyer flask. Add 100 ml of Wulfig's precipitant in such manner as to wash down funnel. Stopper flask, and shake vigorously for 1 min., pausing from time to time to remove stopper and relieve pressure. Let sit overnight. Filter contents thru 12½ cm medium quantitative paper. With wash soln from a wash bottle, wash out flask, pouring washings thru filter; but make no attempt completely to transfer residue. Reserve flask for later determination of acid-soluble constituents. Wash residue on paper thoroly with wash soln. Determine zinc in filtrate as follows: Exactly neutralize to methyl red with HCl , add 200 ml of H_2O , and bring nearly to boiling on hot plate. Add 60 ml of 10% $(\text{NH}_4)_2\text{HPO}_4$, and continue to heat at just below boiling for 30 min. Remove and allow to cool slowly to room temp. Filter thru Gooch crucible that has been tared after ignition for 10 min. at full heat of Fisher burner. Wash with freshly prepared 1% soln of $(\text{NH}_4)_2\text{HPO}_4$, and finally with 50 ml of 50% alcohol. Discard filtrate. Place Gooch crucible in porcelain crucible of suitable size, and dry over low flame. Increase temp. and ignite at full heat to constant weight.

$$\text{Zn}_3\text{P}_2\text{O}_7 \times 0.534 \text{ equals ZnO.}$$

(Talcum powders often contain boric acid. When present, it will accompany ZnO , and must be separated before precipitation of the zinc.)

Acid-Soluble Calcium

Place paper containing residue from the zinc separation in a platinum dish and burn off paper at below 650°C. Transfer to a 250-ml beaker. Use 100 ml of (1+9) HCl to wash residue out of flask used in zinc separation, adding washings to beaker. (If some residue still clings to inside of flask, tilt up at steep angle over beaker, and wash out with stream of water from wash bottle.) Stir thoroly, allow to sit for 10 min and filter thru medium quantitative paper. Disregard turbidity in filtrate, since this will be recovered in the next step. Wash residue on paper three times with distilled water. Place in a platinum dish not less than 6 cm in diam. nor less than 2 cm high, and hold pending addition of recovered acid-soluble Fe , Al , and BaSO_4 . With NH_4OH , nearly neutralize the filtrate to methyl red. Add 200 mg of $(\text{NH}_4)_2\text{SO}_4$ and enough Br_2 water to destroy indicator and distinctly color soln. Boil free of bromine, add more methyl red, and while still nearly boiling add NH_4OH dropwise to the first distinct yellow, avoiding any excess. Let sit for ca 3 min., and filter thru a medium quantitative paper. Wash with hot 2% soln of NH_4Cl . Transfer paper and residue to the platinum dish containing acid-insoluble constituents. Determine calcium in filtrate according to 6.48, beginning with "heat to boiling. . . ."

Acid-Soluble Magnesium

Determine in filtrate from acid-soluble calcium as directed in 37.64 (p. 642).

$$\text{Mg}_2\text{P}_2\text{O}_7 \times 0.3621 \text{ equals MgO}$$

Decomposition of Silicates; Solution of Titanium; Estimation of Barium Sulfate

APPARATUS

Air bath.—On a tripod over Fisher burner place clay triangle having a per side length of about 3 inches. In this triangle set a nickel or iron crucible of about 125 ml capacity, and on top of the crucible set a second clay triangle having a per side length of about 2½ inches. Purpose is to supply controlled radiated heat to platinum dish resting on the top triangle.

METHOD

Ash residues reserved in platinum dish (acid-insoluble portion and materials recovered prior to calcium precipitation) at below 650°C. Pulverize ash with flattened glass rod, and moisten with 4 ml of water. Add 4 ml of H_2SO_4 , place under a hood, and fill dish to ca one-fourth of its depth with HF (48%). Evaporate on the air bath, swirling occasionally to mix contents, until only the H_2SO_4 appears to remain; then cautiously heat over the low flame of a Fisher burner to a pasty consistency. (Do not take to complete dryness.) Add 15 g of pulverized potassium pyrosulfate, and heat to melting. Continue heating, gradually raising temp. until a clear melt is obtained. This will be achieved only when the dish glows red-hot and the melt orange-red. Too rapid heating will cause spattering. Foaming will occur but is not to be feared. At the completion of fusion, the clarity of the melt may be marred by bubbles and possibly by a few flakes of K_2SO_4 produced by the high temp., but these may be disregarded if the melt is generally clear. Set dish aside on an asbestos board and allow to cool. Melt will normally crack away from dish during cooling. Dislodge melt into a 600-ml beaker, wash dish with successive portions of hot (1 + 19) H_2SO_4 until a volume of about 150 ml is obtained, and boil until the melt goes into soln. If present, BaSO_4 comes down at this point. In this event, let digest on a steam bath for one hour, allow to cool, dilute to about 400 ml, stir well, and allow to sit for at least two more hours. Filter thru the finest available quantitative paper, catching filtrate in a 500-ml volumetric flask. Wash thoroly three times with water. Transfer residue to a tared porcelain crucible, burn off paper at low temp., and ignite at dull red heat. Weigh as BaSO_4 . (Residues amounting to less than 0.5% should not be counted as BaSO_4 . Where they occur, they represent HF-resistant silicate or quartz originally present in the talc or kaolin.)

Total Titanium and Iron

APPARATUS

Jones reductor.—Take a 50-ml pinchcock buret (without pinchcock attachment), and with a long glass rod ram down into its constricted lower end a pledget of glass wool. Fill buret to about the 15 ml mark with 20 or 30-mesh amalgamated Zn. (Zn may be amalgamated by letting fall into 200 ml of H_2O containing 4 g of dissolved HgCl_2 and 10 ml of H_2SO_4 . It should be washed several times with distilled H_2O by decantation before being put into buret.) Fit constricted lower end of buret with a 4-inch piece of thick-walled rubber tubing bearing a screw-clamp about the middle and terminating in a glass tube thrust thru a one-hole #7 rubber stopper. The stopper should be fitted to a 500-ml vacuum flask and the glass tube should be of such length as to reach within about 2 inches of the bottom of the flask. When not in use, the Jones reductor should be kept filled with distilled water.

DETERMINATION

Make filtrate from BaSO_4 to volume, pipet into a beaker an aliquot of 100 ml, and add with stirring 5 ml of H_2SO_4 . Place in vacuum flask 10 ml of 10% ferric alum

(free of ferrous Fe and other substances reducing KMnO_4). Fit flask to reductor, apply vacuum, and open screw clamp enough to permit controlled passage of liquid into flask. When meniscus in buret has sunk nearly to level of zinc, add more soln. (It is preferable never to expose amalgamated zinc to the air.) When all of soln has been added, add in the same manner about 100 ml of distilled H_2O . Close screw clamp just before meniscus of last washing reaches level of zinc, release vacuum, and disconnect flask. Transfer contents to 300-ml tall-form beaker and add 3 ml of syrupy phosphoric acid. Using a 10-ml microburet, titrate over a white surface with 0.1 N KMnO_4 to the first pink. Make up a blank containing 3 g of potassium pyrosulfate and 6.5 ml of H_2SO_4 in 100 ml of distilled H_2O . Put this thru identically the same treatment the sample received, finally titrating to the same shade of pink. Subtract titre of blank from that of sample. Corrected titre $\times .008$ equals total (TiO_2 plus Fe_2O_3) (these have practically the same equivalent weight).

Total Iron

REAGENT

Titanium trichloride, 0.05 N TiCl_3 .—Make up according to directions in 21.36 (p. 290), but containing only half as much TiCl_3 as required for the 0.1 N soln. Standardize according to either of the methods listed under 21.37 except that standardization should be conducted using an ordinary micro-buret and titrating into an open beaker. The soln should be kept in an ordinary glass-stoppered bottle and re-standardized immediately before each set of determinations.

DETERMINATION

Pipet an aliquot of 100 ml from volumetric flask into a 150-ml beaker. Add 1 g NH_4CNS . Slowly and with thoro stirring, titrate with 0.05 N TiCl_3 from a microburet to disappearance of the red color. Run a blank on 3 g of potassium pyrosulfate and 6.5 ml H_2SO_4 in 100 ml of distilled H_2O . (Blank is often nil.) Corrected titer $\times .004$ equals Fe_2O_3 .

Total Titanium

Per cent total (TiO_2 plus Fe_2O_3)—per cent total Fe_2O_3 equals per cent total TiO_2 .

Total Oxides of Iron, Titanium, and Aluminum

Pipet an aliquot of 250 ml from volumetric flask into a 600-ml beaker. Add a few drops of methyl red indicator and 5 g of NH_4Cl , and bring to boil. Neutralize by adding NH_4OH dropwise just to the first distinct yellow. Let sit for about 3 min., and filter thru a 12½ cm medium quantitative paper. Wash several times with hot 2% NH_4Cl . Place paper in a tared crucible and dry in an oven or an air bath. Transfer to a muffle furnace at room temp., and raise heat to about 1100 degrees. Ignite to constant weight. Result is total (Al_2O_3 plus Fe_2O_3 plus TiO_2).

Total Aluminum

Per cent total (Al_2O_3 plus Fe_2O_3 plus TiO_2)—per cent total (Fe_2O_3 plus TiO_2) equals per cent total Al_2O_3 .

Acid-Insoluble Calcium

Determine calcium in the filtrate from the ammonium hydroxide precipitate according to directions in 6.48 (p. 66), beginning with "heat to boiling. . . ."

Acid-Insoluble Magnesium

Determine in filtrate from acid-insoluble calcium by 37.64 (p. 642). $\text{Mg}_3\text{P}_2\text{O}_7 \times 0.3621$ equals MgO .

Silica

Weigh about 1 g of the powder into a 250-ml beaker. Moisten with alcohol and add 100 ml of (1+9) HCl. Stir, and allow to stand for 10 min. Filter thru 12½ cm medium quantitative paper. Wash the residue 3 times with H₂O. Transfer paper to a platinum crucible and ash at below 650°C. Cool, and pulverize ash with a flattened glass rod. Add 6 g of Na₂CO₃ a portion at a time, intimately mixing with the same glass rod between additions. Use the last of the Na₂CO₃ to sprinkle over the top of the mixture. Place in a muffle furnace at below 800°C., and raise temp. to bring contents into fusion. Heat at ca 1000°C. for 15 min. Remove the crucible and let cool. Dislodge the melt into a dry 500-ml beaker. (Dislodging the melt is not always easy. It often helps to return the crucible to the hot furnace for ½ min., then remove it and immediately dip about two-thirds of its length in a beaker of H₂O. If repeated a sufficient number of times, this treatment causes the melt to crack away from the platinum so that it can be removed by simply upending the crucible over the beaker.) In a graduate mix 15 ml of HNO₃ with 5 ml of H₂O, and wash the crucible with small successive portions of the mixture, adding washings to the beaker. If soln of the melt becomes slow, hasten its disintegration by gentle pressure with a glass rod. When the Na₂CO₃ in the melt has dissolved, place the beaker under a hood and add, in the order named, 5 g of NH₄Cl and 25 ml of HClO₄ (60%). Cover the beaker with a watch glass, and boil over a moderate flame until oxides of nitrogen have passed off and the HClO₄ refluxes down the sides of the beaker. Cool the mixture slightly, add 150 ml of very hot water, stir, and let sit until silica settles to the bottom. Decant supernatant liquid thru a 12½ cm medium quantitative paper, and transfer residue to paper using hot water and policing out beaker. Wash thoroly five times with hot water. Transfer to a platinum dish, burn off paper, and ignite to constant weight at about 1100°C. Weigh as crude silica. To the residue in the dish add ca 2 ml of (1+9) H₂SO₄ and enough HF (48%) to cover the silica. Heat on a steam bath under the hood until silica and excess HF have passed off. Cautiously heat over the non-reducing flame of a Fisher burner until fumes of SO₂ have ceased to be evolved, and then heat strongly for several min. Cool and reweigh. The difference between this weight and the weight of crude silica is weight of SiO₂.

Starch

Weight ca 5 g of the powder into a 500-ml Florence flask (preferably standard taper). Moisten with 10 ml of alcohol. Acid-wash according to directions in 17.20 (p. 212), hydrolyze starch as directed under 27.33 (but filter hydrolyzed mixture before and not after making to volume), and determine dextrose by 34.39 and 34.40 (p. 572).

5. ENZYMES

No additions, deletions, or other changes.

6. ECONOMIC POISONS

(Formerly Insecticides and Fungicides)

(1) The tentative methods for DDT, *This Journal*, 30, 64-66 (1947) as amended, and *Ibid.*, 31, 73 (1948) were adopted as official, first action.

(2) The tentative method for DDT, *This Journal*, 31, 72 (1948) entitled "Total chloride in emulsions containing DDT, Solvent, Emulsifying Agent, and Water" was adopted as official, first action.

7. CAUSTIC POISONS

No additions, deletions, or other changes.

8. NAVAL STORES

No additions, deletions, or other changes.

9. GELATINE, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or other changes.

10. LEATHERS, AND 11. TANNING MATERIALS

The methods in Chapters 10 and 11 were deleted.

12. PLANTS

No additions, deletions, or other changes.

13. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

14. MALT BEVERAGES, SIRUPS, AND EXTRACTS, AND BREWING MATERIALS

(1) The tentative method for color determination of beer and wort, *This Journal*, 30, 68 (1917), was deleted.

(2) The following method was adopted as official, first action.

DYE COLOR METHOD*

REAGENTS

(a) A dye color stock soln is prepared by dissolving 0.2636 g Amaranth #184, FD&C Red #2; 0.6948 g Tartrazine #640, FD&C Yellow No. 5, and 0.0487 g Patent Blue, V.F., Extra Concentrated, in 200 ml of distilled water. Add 200 ml of methanol and make up to 1 liter in a volumetric flask. Transfer the soln to an amber, glass-stoppered bottle and store in a dark place. Under these conditions the soln is stable indefinitely.

(b) *Mercuric chloride*, saturated soln (ca 80 g per liter).

METHOD I

To prepare the various Dye Color Reference Solutions, add by buret the volume of stock soln in ml corresponding to the Dye Color Value (Lovibond Equivalent) desired. For the range 1° to 5° dye color values in 0.25° intervals, use 1 ml, 1.25 ml, 1.5 ml, etc., each diluted to 500 ml in a volumetric flask. Before the final volume is reached, add 10 ml of mercuric chloride soln to each flask as a preservative. After thoro mixing, pour these dye color solns into properly labeled 4 oz. comparison bottles (Owens-Illinois #A-4906 is suitable type) and keep in a closed box or covered comparison rack.

METHOD II

This alternate procedure may be used to advantage in most instances.

* The complete apparatus, stock soln, bottles, and dyes can be obtained from the Fisher Scientific Co 2109 Locust St., St. Louis 3, Mo. The dyes can also be obtained from American Society of Brewing Chemists, 64 E. Lake St., Chicago, Ill. For complete description of the method and apparatus, see *This Journal* 29, 287 (1917).

By means of the buret, add 12 ml of the concentrated stock soln to a 1-liter volumetric flask and add distilled water to volume. This produces a 6° dye color stock soln which is further diluted as follows to make up the necessary lower values.

°Dye Color

Dye Color Reference Solutions.....	1, 1½, 1½, 1½, 2, 2½, 2½, 2½, 3,
Vol. (ml) 6° Stock soln/120 ml.....	20, 25, 30, 35, 40, 45, 50, 55, 60,
Dye Color Reference Solutions.....	3½, 3½, 3½, 4, 4½, 4½, 4½, 5
Vol. (ml) 6° Stock soln, 120 ml.....	65, 70, 75, 80, 85, 90, 95, 100

(For the color of beer, the first four values are usually not required. It is customary, therefore, for beer color sets, to prepare the colors from 2° to 5°, instead of beginning with the 1° soln.)

Add, by buret, the above indicated volumes of 6° stock soln, to labeled 4 oz. comparison bottles. Add 5 ml of saturated mercuric chloride soln to each and make up to 120 ml by adding distilled water in appropriate volume from a buret. For example, the 3° dye color soln contains 60 ml of 6° stock soln, 5 ml of mercuric chloride soln, and 55 ml of distilled water. The finished dye color solns are kept in a closed box.

DETERMINATION

Prepare the sample by filtering thru Reeve Angel #202, 15 cm paper using a small amount of filter-cel. Occasionally a double filtration is necessary. Filtration does not affect the color.

Pour the prepared sample into a comparison bottle and match with the Dye Color Reference Solutions against a source of constant, diffused light. For example, a white blotter illuminated by a frosted light bulb or fluorescent light will serve. Matching should be done by placing the sample between consecutive dye color bottles and viewing them thru the longer axis of the bottle. Repeat until the proper pair is found. It is often possible to interpolate a value between consecutive reference solns. Report the color in terms of degrees dye color, which is equivalent to °Lovibond, ½° Cell, Series 52.

(3) The tentative method for total solids in yeast, 14.112-14.115, incl. (p. 178) was adopted as official, first action, with the following revision of 14.114.

SAMPLING

14.114

(a) Collection of primary sample—

(1) *Liquid yeast*.—From small tanks and tubs mix contents well to uniform consistency, taking care to blend in the heavier deposit on bottom of vessel. Take ca 500 ml as sample in cold glass container which will hold at least twice actual sample volume.

From larger tanks or those whose contents may not be mixed, take at least a 4-liter composite sample of separate aliquots taken from hose line as yeast is transferred, or by use of a trier device which will take samples from all levels in the tank. Mix this composite sample to homogeneity, then take the smaller sample from this, as above.

(2) *Pressed yeast*.—Remove portions from different parts of surface as well as center of cake and collect ca 150 g in 1000 ml beaker. Weigh to nearest 0.1 g. Prepare slurry by adding H₂O at rate of ca 3 parts of H₂O/1 part of pressed yeast. Again weigh to nearest 0.1 g. With stirring rod break up yeast portions and stir until completely uniform liquid suspension is obtained.

(b) *Care of sample*.—To prevent changes in analytical results due to autolysis and fermentations, proceed with examination of yeast samples immediately after the samples have been obtained.

(c) *Preparation of sample*.—Mix well and stir until complete uniform suspension is obtained. If lumps or particles of trub are present, pass the entire bulk of the primary sample thru sieve of ca 100 mesh. Make sure that all lumps and particles, including trub, are broken up and forced thru the sieve.

Remove, by scraping, any liquid or solids adhering to the sieve and reincorporate with the sieved sample. Mix well by stirring, then withdraw aliquot for separate determinations.

(4) The official Milos test for caramel, 14.35 (p. 158) was deleted, final action (first action, *This Journal*, 31, 75 (1948)).

15. WINES

(1) The official Milos test for caramel 15.38 (p. 188) was deleted, final action (first action, *This Journal*, 31, 75 (1948)).

(2) The official, first action, Mathers test for caramel, *This Journal*, 31, 76–77 (1948) was made official, final action.

16. DISTILLED LIQUORS

(1) The official modified Marsh test for caramel 16.39 (p. 202) was deleted, final action (first action, *This Journal*, 31, 77 (1948)).

(2) The official Milos test for caramel 16.41 (p. 202), was deleted, final action (first action, *This Journal*, 31, 77 (1948)).

(3) The official, first action for caramel, *This Journal*, 31, 77(1948), was made official, final action for distilled liquors.

(4) The following method was adopted as official, first action.

PROOF OF DISTILLED SPIRITS (RAPID METHOD)

Determine the apparent proof of the distilled spirits with an accurately standardized hydrometer, preferably one graduated in $\frac{1}{2}$ degree in proof. Determine the extract (solids) according to section 16.8 and for every 100 mg extract add 0.4° proof to the apparent proof. NOTE: If the extract amounts to more than 600 mg this method does not apply.

(5) In the method for specific gravity of Cordials and Liqueurs, 16.45 (p. 203), "proceed as under 16.2" was substituted for "see 14.3."

17. BAKING POWDERS AND BAKING CHEMICALS

(1) The tentative modified McGill method for starch, 17.21 (p. 212) was deleted.

(2) The tentative qualitative test for phosphoric acid, 17.31 (p. 214) was adopted as official, first action.

(3) The tentative method for residual carbon dioxide, *This Journal*, 31, 78 (1948), was adopted as official, first action.

(4) In 17.4 and 17.6 (pp. 208 and 209), HCl (1+2) was adopted as alternate for H₂SO₄ (1+5).

(5) The following method was adopted as official, first action.

RESIDUAL CARBON DIOXIDE

DRYING OVEN METHOD

Place 1.7 g of baking powder in clean, dry 250 ml wide-mouthed Soxhlet extrac-

tion flask (flask A 17.6). Tap flask to spread sample evenly on bottom. Add 10 ml of H_2O with a pipet. Stir with a glass rod to break up powder which may have caked on bottom of flask. Wash down stirring rod and sides of flask with 10 ml H_2O . Place the flask into an air drying oven set to maintain a temp. of $100^\circ C. \pm 2^\circ$, on a shelf near the center of the oven and evaporate to dryness. After 5 hours remove from the oven, add 10 ml H_2O and cool to the same temp. as the air surrounding the Chittick apparatus. Determine CO_2 present in residue with Chittick apparatus 17.6, using correction factors in 44.30. Shake the flask vigorously until further shaking produces no increment in reading.

(6) The official method for available carbon dioxide 17.9 (p. 210) as revised, *This Journal*, 31, 78 (1948) was changed to read "Subtract residual CO_2 , 17.7, from total CO_2 , 17.3; or subtract residual CO_2 by gasometric method, *This Journal*, 31, 78 (1948), or by the drying oven method (see change No. 5, above) from total CO_2 , 17.6."

(7) The official gasometric method for residual carbon dioxide, 17.8 (p. 210) was deleted, final action.

18. COFFEE AND TEA

(1) The Fendler-Stüber modified method for caffeine in coffee, 18.15 (p. 217) was adopted as official, final action (first action, *This Journal*, 30, 70 (1947)).

19. CACAO BEAN AND ITS PRODUCTS

(1) In the second paragraph of 19.16 (p. 227) of the tentative method for pectic acid, line 5, beginning "Neutralize" to the end of the paragraph, was deleted and the following substituted:

"Neutralize to litmus with NH_4OH (1+1) (ca 1 ml), then make slightly acid with acetic acid and add 50 ml of 2% NH_4 oxalate soln. Place power driven glass rod stirrer with a vertical loop at the end in the flask with the shaft thru tube inserted in a No. 10 rubber stopper. Place flask in water bath held at $90-92^\circ C.$ and stir contents gently and continuously for three hours."

20. CEREAL FOODS

(1) The official, first action, methods for phosphorus in cereals and cereal products, *This Journal*, 31, 79 and 80 (1948) were adopted as official, final action.

(2) The tentative method for acidity in flour and grain, 20.18-20.21, incl. (pp. 241 and 242) and 20.76 (p. 260) were adopted as official, first action.

(3) The tentative method for benzoyl peroxide bleach in flour, 20.53 (p. 253) was deleted. (Method retained for rye flour.)

(4) The following method was adopted as official, first action.

BENZOIC ACID IN FLOUR (WHEAT)

QUALITATIVE TEST

Place 50 g of flour in (preferably) 500 ml glass-stoppered flask, add 30-40 glass beads (about 6 mm diam.), 0.1 g powdered iron, 100 ml ether (isopropyl ether, which is cheaper and less volatile, may be used). Allow to stand few min., shake with rotary

motion and add slowly (preferably dropwise) 2.5 ml HCl from Mohr pipet. Allow to stand overnight. Shake well with rotary motion, allow flour to settle a few minutes and decant thru Büchner funnel (100 mm) fitted with filter paper moistened with ether, into 500 ml suction flask. Add 50 ml ether, shake and allow to settle a few min., decant as before, repeat twice more, transferring the whole contents to funnel following last addition. Transfer thru large funnel into 250 ml separatory funnel, add 20 ml 5% NaHCO_3 soln., mix without too much vigorous shaking, and draw off the lower clear layer into 125 ml Erlenmeyer flask, repeat with one more 20 ml portion and two 10 ml portions of 5% NaHCO_3 . Add to this soln 0.3 g Nuchar W, shake and filter (11 cm 589 white ribbon, S&S or equivalent) into 200 ml Erlenmeyer flask, wash flask, and filter with about 20–25 ml H_2O , using fine stream from wash bottle, add 2.0 ml H_2SO_4 (1+1) dropwise to avoid foaming out of flask. Swirl contents gently to reduce foaming. (The soln should be definitely acid to litmus paper.) Transfer to 125 ml separatory funnel, rinse flask with 12 ml ether and add to funnel, shake gently with frequent release of pressure due to ether and CO_2 . (During first extraction with ether, it is preferable to release pressure after each shake, to avoid possibility of loss.) Repeat with two more 12 ml extractions with ether. Rinse flask each time with ether. After each extraction draw off aqueous soln into the same 200 ml Erlenmeyer flask and transfer ether to a Pyrex test tube (ca 50 ml capacity, 25 mm diam. and 150 mm length). Add 2 ml 10% NaOH, hold top of tube firmly against the palm of the hand and shake vigorously, insert piece of copper wire (1 mm diam. \times 200 mm) into tube, evaporate the ether very slowly on steam bath. Remove copper wire. Then place tubes into beaker of boiling water and evaporate to nearly dryness, add slowly up to 0.5 ml 30% H_2O_2 , followed by another 0.5 ml as soon as foam condition permits. (Minimum frothing is desirable to permit better contact for nitration.) Continue evaporation until there is no apparent moisture in tube. (The introduction of a gentle air blast into the tube hastens evaporation.) Add from Mohr pipet 4 ml of (1+1) mixture of H_2SO_4 and fuming nitric acid, taking care to have it wash down the sides of tube, heat 20 min. in gently boiling water bath (occasionally rotate or mix to ensure contact with nitrating mixture) immediately cool under tap to below room temperature, add 6 ml water while continuing to keep cool. Then *slowly* add 5 ml conc. NH_4OH by means of a Mohr pipet with continuous shaking under the tap to keep soln cool. Add 10 ml more of conc. NH_4OH keeping soln continuously cool. Add 2 ml of 6% hydroxylamine hydrochloride soln, stir, place in 65°C. water bath 5–6 min., stirring occasionally. (Temperature of bath should be a few degrees above, since the cold tubes cause some decrease.) Cool to room temp. under tap, filter immediately thru folded filter into similar tube and observe color of filtrate. Red or definitely pink color indicates the presence of benzoic acid.

Immediately transfer this soln (within 30 min.) to a 2 in. glass cell and read in a neutral wedge photometer, using No. 51 filter or in other equally precise instrument at wave length 510. Standardize the instrument by placing in test tubes 0.0, 0.4, 0.8, 1.0 and 1.2 mg benzoic in acetone soln (0.5 mg to 1 ml). Add 2 ml 10% NaOH, shake to mix well, and proceed as above beginning "place tubes into beaker of boiling water." Report individual results in p.p.m. of benzoic acid.

(5) The tentative method for fat and fat number of bread 20.86 (p. 261) was deleted.

(6) The following method was adopted as official, first action.

FAT AND FAT NUMBER OF BREAD

Slice one loaf of bread, and allow to dry overnight, or until sufficiently dry to grind. Grind bread to ca size of openings on 20-mesh sieve, mix, sample, and transfer 50 g to 600 ml beaker. Add 100 ml of H_2O and mix. Add 100 ml of HCl, mix, cover and heat on steam bath 1 hour, stirring well 6 or 7 times. Cool in cold (15° or less)

water bath, and stir. Add 10 g of Filter Cel, or other similar adsorbents, stir, and mix completely. Prepare 90 mm Büchner funnel as follows:

Place two No. 590 S&S 9 cm filter papers (or equivalent) in funnel and apply suction. Mix 10 g of Filter Cel with 50 ml of H_2O and rapidly pour mixture into funnel. (This should make a smooth even layer of Filter Cel over filter paper, with no cracks or openings.) Filter sample immediately. Rinse out beaker several times with ice cold H_2O . Just before filtration is complete, wash down sides of Büchner with ca 100 ml of ice-cold H_2O (or until clear filtrate comes thru). Up to this point do not allow pad to suck dry. Continue with suction until Filter Cel pad seems dry. Transfer this mass, without filter paper, from Büchner to original beaker. Break up mass with rod, dry (overnight) on steam bath and then heat in oven at 100° ca $\frac{1}{2}$ hour to remove all moisture (material must be dry or fat results will be low). Break up any lumps. Prepare large Knorr extraction tube of ca 200 ml capacity (glass tubing 5 cm in diameter with height of 12 cm from shoulder to top of tube). Pack tube with asbestos tamped tightly to form pad ca $\frac{1}{4}$ " thick. Insert stem of tube into 2-holed rubber stopper in filtering bell jar connected to suction thru 2-way stopcock. Place 500 ml Erlenmeyer flask within bell jar so that stem of tube passes thru neck of flask. To cool beaker and contents, add 100 ml of mixed ether and petroleum benzine (1+1) and macerate against sides of beaker with medium-sized stiff metal spatula 3-4 min. Decant into extraction tube. Suck dry. Add to beaker 80 ml of the mixed ethers. Work as before 2 min. Transfer contents of beaker to extraction tube, suck dry, and tamp with flattened stirring rod until all ether is removed. To material in tube add 80 ml of the mixed ethers that have just previously been used to rinse out beaker, mix thoroly with stirring rod a few min., allow to stand a min., then suck dry, and tamp material as before. Make two additional extractions, turning suction on and off carefully to avoid loss of sample in Erlenmeyer flask. Transfer to 1 liter beaker. Evaporate on steam bath, completely transfer fat with small amounts of petroleum benzine to tared 150 ml beaker, carefully evaporate benzine on steam bath, dry at 100° to constant weight (ca 30 min.), cool, and weigh. Calculate percentage of total fat on moisture-free basis.

Weigh duplicate samples of 1 g (within $\pm .03$ g) of fat into 300 ml Florence flasks, add 4 ml of glycerol-soda solution, 31.28(c). Heat the flask carefully over asbestos gauze until bubbles start to appear, then hold the flask about an inch over the heated gauze until cloudiness or turbidity disappears, and a perfectly clear mixture is obtained. After the mixture first becomes clear, $\frac{1}{2}$ or 1 min. more gentle heating insures complete saponification. Cool, add few pieces of previously ignited pumice stone, 138 ml of CO_2 -free H_2O , and 3 ml of H_2SO_4 (1+4), and proceed as directed in 31.29, using same apparatus. Use 0.02 N NaOH for titration. Multiply ml of 0.02 N NaOH used by 1.1 and divide by weight of fat used. Run blank determination and make correction. Report number of ml 0.02 N NaOH per 1 g of fat as "fat number."

(7) The following method was adopted as official, first action.

PROTEOLYTIC ACTIVITY OF FLOUR AND MALTED WHEAT FLOUR

(Applicable to slightly active materials such as patent flour or to diluted extracts of active proteolytic preparations.)

REAGENTS

(a) *Buffer stock soln.*—Make 120 ml of acetic acid and 164 g anhydrous sodium acetate up to 1000 ml with H_2O . Dilute 1:20 before using (pH 4.7).

(b) *Bacto-hemoglobin substrate.**

(c) *Trichloroacetic acid solns:*

* A suitable quality is obtainable from the Difco Laboratories, Detroit, Mich.

Soln (1).—Dissolve 36 g trichloroacetic acid in 64 ml water. Use 5 ml aliquot.

Soln (2).—Dissolve 36 g trichloroacetic acid in 44 ml water. Use 4 ml aliquot.

(d) *Kjeldahl solns.*—Including 0.0714 *N* sodium hydroxide.

(e) *Pumice or fine sand.*

METHOD

Preparation of enzyme solutions.—For slightly active materials such as flour, weigh as much as 10 g directly into digestion flasks. For active enzyme preparations prepare a water extract or suspension immediately preceding digestion. (The amount of extract or dilutions thereof used in the digestion mixture may vary up to 1 ml. Appropriate activation technics may be applied to the enzyme extract.)

Digestion procedure.—Weigh 0.625 g (moisture-free basis) of Bacto-hemoglobin into each 125 ml Erlenmeyer flask and add ca 3 g of finely divided pumice. Add 5 g sample of flour to each of two flasks and agitate the mixture by rotation until flour and substrate are intimately mixed. Then add 25 ml of reagent (a) previously warmed to 40° (in thermostat-controlled bath within $\pm 0.1^\circ\text{C}.$) to each flask and agitate its contents to insure uniform suspension. Place the tightly stoppered flasks in a constant temp. (40°) bath and agitate either continuously or at hourly intervals.

Add a 5 ml portion of trichloroacetic acid soln c(1) to one flask of each pair at the end of 15 min. digestion and to the second flask of the pair after 5 hours of digestion. Mix contents thoroly and allow flasks to remain in the bath at 40°C. for exactly 30 min. Centrifuge the suspension for 5 min. at 1800 r.p.m. and filter.* Pipet duplicate 5 ml aliquots directly into Kjeldahl flasks and determine soluble nitrogen.

Follow essentially the same procedure in determining the enzyme activity of an extract. In place of the solid material, use a total of 1 ml of extract or extract plus water. After zero time and 5 hour digestion periods, add to each flask a 4 ml aliquot of trichloroacetic acid soln c(2). Thoroly mix the contents, allow to remain in the water bath for exactly 30 min. and filter without centrifuging; analyze 5 ml aliquots for soluble nitrogen.

Soluble nitrogen.—Proceed as under 2.24, 2.25, or 2.26. Use a definite volume of water (350 ml) to dilute the cooled digest and add in such a way as to wash down all the trichloroacetic acid which has condensed in the neck of the flask during the digestion process. Also add the concentrated alkali (one and one-half times the usual quantity) in such a manner as to lave the neck of the flask. After distillation, back-titrate the unneutralized standard acid with 0.0714 *N* sodium hydroxide.

Expression of proteolytic activity.—Proteolytic activity is measured by the difference in back-titration volumes for the 15-min. or zero time digestions and the corresponding 5 hour digestion, expressed in ml of 0.0714 *N* sodium hydroxide. This difference may be translated into mg of soluble nitrogen released from a given weight of the enzyme source.

(8) The tentative method for moisture of soybean flour, 20.77 was changed to read "Proceed as under 20.4, using a 5 g sample and drying at 130° for 2 hours," and adopted as official, first action.

(9) The tentative methods for ash 20.78, nitrogen 27.79, and oil or petroleum benzine extract 20.82 of soybean flour, as revised, *This Journal*, 31, 81 (1948), was adopted as official, first action.

(10) The following method was adopted as official, first action.

* Some materials such as flour may remain turbid after the final filtration. Such turbidity may be removed by boiling the centrifuged digestion mixture for a few seconds prior to final filtration. The liquid lost through evaporation should be replaced by the addition of water.

ADDED INORGANIC MATERIAL IN PHOSPHATED FLOUR

QUALITATIVE TEST

Transfer 20 g of flour to dry 250 ml separator, add ca 200 ml carbon tetrachloride, shake well, let stand until solution at bottom is nearly clear, usually ca 15 min. Draw off sediment with a minimum of solution, by turning the stop-cock quickly from side to side, into dry 125 ml separator containing about 100 ml carbon tetrachloride. Again shake 250 ml separator, let stand, with occasional gentle swirling, if necessary, to dislodge sediment from separator sides, until lower portion of soln has cleared. Draw off sediment from 125 ml separator into prepared and weighed Gooch crucible, using suction. Draw off sediment from 250 ml separator into 125 ml separator as before, and let stand with occasional gentle swirling to dislodge sediment from the sides of the separator. . . . When lower portion of liquid has cleared, draw off into Gooch crucible as before. (Care must be taken that no sediment remains on ledge in separator.) Wash the crucible and contents with 25 ml fresh carbon tetrachloride, continue aspirating 2 or 3 min. and weigh at once. Ignite crucible containing the sediment obtained above at 700°C., cool, and weigh as calcium metaphosphate. Weight of calcium metaphosphate, $\text{ca } (\text{PO}_3)_2 \times 1.27 \times 5 = \text{Percentage of monocalcium phosphate monohydrate, ca } (\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ in the flour.

(11) The tentative methods for separation of proteins, 20.32-20.38, and the tentative method for detection of rye flour in wheat flour, 20.60, were dropped.

21. COLORING MATTERS

(1) The tentative rapid method for tartrazine, 20.125 (p. 268) as revised and adopted as official, first action, *This Journal*, 31. 82 (1948), was adopted as official, final action.

(2) The following method was adopted as official, first action.

LEAD IN COAL-TAR COLORS

(Not containing calcium, barium, or strontium.)

REAGENTS

- (a) *Standard lead soln.*—*Methods of Analysis*, A.O.A.C., Sixth Edition, 29.36(a).
- (b) *Nitric acid soln*, 1%.—*Ibid.*, 29.36(b).
- (c) *Citric acid soln*, 50%.—(*Special grade—low in lead*) *Ibid.*, 29.36(d).
- (d) *Diphenylthiocarbazone (dithizone) soln.*—Stock soln of purified dithizone containing (100 mg. per ml.) chloroform. A working soln containing 20 mg. per liter. *Ibid.*, 29.36(e).
- (e) *"Stripping" reagent.*—*Ibid.*, 29.36(f).
- (f) *Potassium iodide soln*, 2%.—*Ibid.*, 29.36(g).
- (g) *Starch soln*, 0.5%.—Weigh 1 gm of soluble starch. Make into a thin paste with several ml of cold water, pour into 200 ml of hot water, and while still hot add 2-3 small crystals of HgI_2 as preservative.
- (h) *Sodium thiosulfate soln.*—Make a stock 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ soln. Use 5 ml isoamyl alcohol per liter as preservative. Prepare daily a fresh 0.001 N dilution and standardize against standard lead solution.
- (i) *Potassium cyanide soln*, 10%.—Dissolve 50 gm. phosphate-free KCN in distilled water and dilute to 500 ml.
- (j) *Hydroxylamine hydrochloride soln*, 10%.—Dissolve 10 gm $\text{NH}_2\text{OH} \cdot \text{HCl}$ in 20 ml of water and make slightly alkaline with ammonia. Extract lead with dithizone. Remove excess dithizone with chloroform and boil off any CHCl_3 remaining in the aqueous phase. Acidify with HCl and dilute to 100 ml.

(k) *Thymol blue indicator soln, 0.1%.*—Dissolve 0.1 gm thymol blue in distilled water, add sufficient 0.1 *N* NaOH to change the dye to blue and dilute to 100 ml.

DETERMINATION

Transfer a 5.00 gm sample to a 500 ml Kjeldahl flask, add 10 ml concentrated H_2SO_4 and 10 ml concentrated HNO_3 , and heat. When evolution of SO_2 fumes begins add 5 ml of concentrated HNO_3 and heat until SO_2 is again evolved. Repeat the additions of concentrated HNO_3 each time SO_2 fumes appear until the dye is completely in solution and the digest is yellow. Then add 10 ml of 1:1 mixture of concentrated HNO_3 and 60–70% HClO_4 ; continue heating until the wet ash is colorless or pale yellow and the bulk of the H_2SO_4 is evaporated.

Cool the flask under running water and neutralize the soln by additions of small portions of concentrated NH_4OH . Add 20 ml of the citric acid soln and then adjust to pH 8.5–9 with NH_4OH using four drops of thymol blue indicator. Add 5 ml of 10% KCN soln.

Transfer the alkaline soln to a 250 ml separatory funnel. Extract the lead with a 20 ml portion of dithizone solution containing 20 mg per liter. (Note: If there is sufficient iron present to cause excessive oxidation of the dithizone as indicated by a yellow color in the CHCl_3 layer, 10 ml of 10% $\text{NH}_2\text{OH} \cdot \text{HCl}$ should be added to reduce the iron.) Allow the chloroform layer to settle and draw off into another separatory funnel. Wash down the floating globule of chloroform with two successive 5 ml portions of weak dithizone (4 mg per liter) and add to the receiving funnel. Repeat the extractions with the stronger dithizone until no more of the red lead dithizonate is observed. Do two more extractions with 10 ml portions of the weaker dithizone soln.

Wash the chloroform extract with 25–30 ml of distilled water containing one drop of concentrated NH_4OH . Draw off cleanly the washed chloroform layer into a third separatory funnel. Add 110 ml. of 1% HNO_3 and shake for one minute. Draw off and discard the chloroform and about 1 ml of the acid layer. Insert a cotton plug into the stem of the funnel to filter the acid layer as it is withdrawn. Discard the first 3 ml of the filtrate. Electrolyze a 100 ml aliquot of the filtrate as directed in 29.41, *Methods of Analysis, A.O.A.C.*

(4) The following methods were adopted as official, first action.

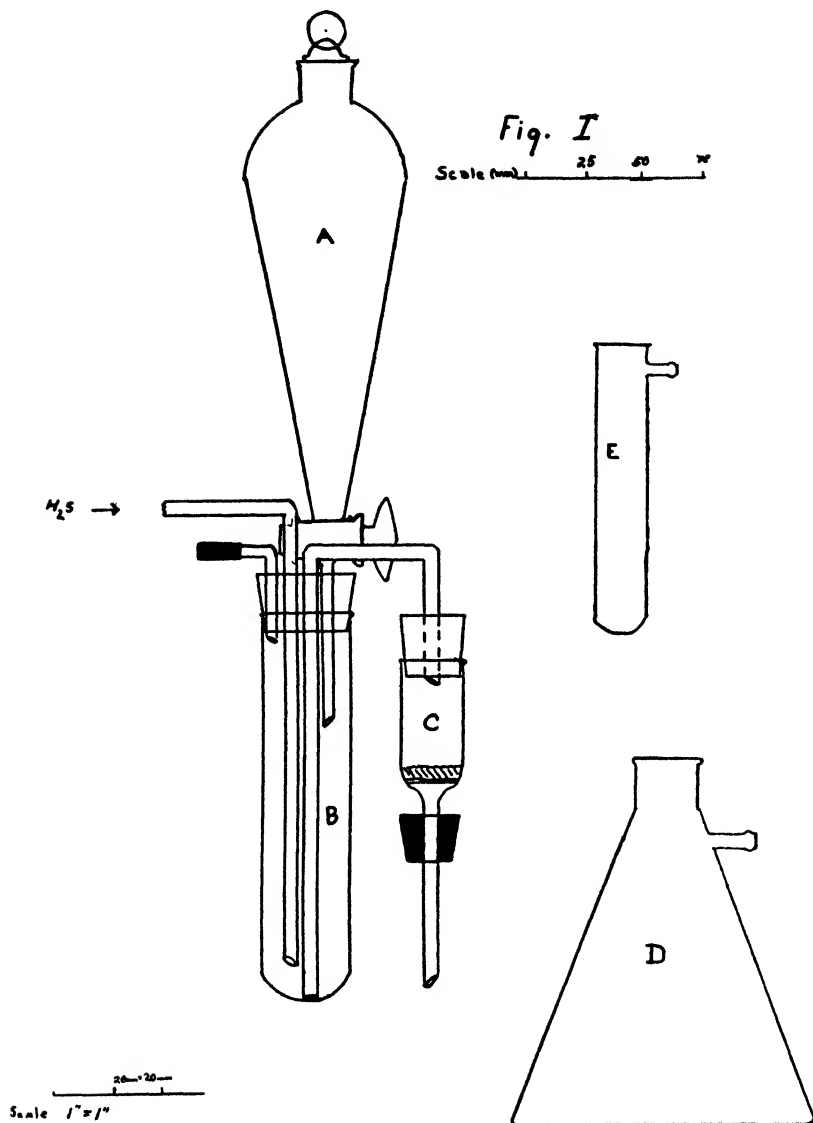
LEAD IN LAKES (ALUMINUM) OF COAL-TAR COLORS

REAGENTS

All reagents should be lead free (29.36). Any convenient source of H_2S may be used. The gas should be scrubbed first with (1:1) H_2SO_4 (v/v), then with distilled water, before being passed into the solution.

METHOD

Weigh 2 g of the sample into a 500 ml Kjeldahl digestion flask, add 10 ml of conc. H_2SO_4 and 10 ml of conc. HNO_3 , and digest on a low flame until SO_2 fumes appear. Add successive 5 ml portions of conc. HNO_3 (waiting until SO_2 fumes appear before adding each succeeding portion) until all organic matter is in solution. Slowly introduce 5–10 ml of a (1:1) mixture of conc. HNO_3 and 60–70% HClO_4 , and continue the digestion until the white ppt. formed showed the first signs of spattering. Allow the flask to cool and cautiously add 5 ml of H_2O and then a few drops of conc. NH_4OH . Swirl the flask vigorously and cool under running water. Add 20 ml of 50% (w/v) citric acid soln and adjust the pH to 3.0–3.4 (bromophenol blue) with conc. NH_4OH . Add 1 ml of CuSO_4 soln containing 1 mg Cu per ml and transfer the soln to the pptn tube (B) of the sulfiding apparatus. (See Fig. I) Bubble H_2S thru the soln at a



rate of ca 2 bubbles per sec. for 3-5 min. and filter the resulting suspension thru (C) at a rate of ca 1 drop per sec. When filtration is complete remove the receiver containing the filtrate and attach a suction test tube as shown in (E). Add 3 ml of hot conc. HNO_3 thru the separatory funnel (A) and draw thru the filter, followed with 2 ml of hot water. Detach the filter and pass an additional 3 ml of hot conc. HNO_3 thru the filter, wetting all sides. Again follow with 2 ml of hot water. If the filter is still colored with PbS , wash again with hot conc. HNO_3 and water. Wash the dissolved sulfides into the pptn tube (B), wetting all sides to take up any residual lead sulfide and then into a 50-100 ml glass-stoppered conical flask. Stopper and shake for a few sec., then remove the stopper and boil until the soln clears, to remove the last traces of H_2S and to coagulate any free sulfur present.

Transfer the soln to a 250 ml separatory funnel. Wash the flask with two 5 ml portions of distilled water and add the washings to the main soln. Add 10 ml of 50% (w/v) citric acid soln, 5 ml of 10% sodium cyanide soln, a few drops of hydroxylamine hydrochloride soln to prevent oxidation of the dithizone, adjust the pH to 8.5-9.5 (thymol blue) with conc. NH_4OH and proceed with the dithizone extraction and electrolysis as directed, 29.39, 29.40, 29.41.

LEAD IN LAKES (CALCIUM, BARIUM, AND STRONTIUM) OF COAL-TAR COLORS

REAGENTS

Sodium carbonate.—Lead free, analytical grade.

Potassium carbonate.—Lead free, analytical grade.

Sodium nitrate.—Lead free, analytical grade.

Sodium carbonate soln.—5% (w/v).

Hydrochloric acid.—(2:5) (v/v).

METHOD

Place 2 g of the lake, 4 g of Na_2CO_3 , 6 g of K_2CO_3 , and 0.5 g of NaNO_2 in a platinum crucible of suitable size. Mix thoroly. Heat carefully until the color is carbonized, then heat to about 850°C . and hold at that temp. for 15 min. If a controlled muffle furnace is available, it is only necessary to place the fusion mixture in the cold furnace and raise the temp. gradually to 850°C . over a two-hour period. Usually 15-30 min. heating at 850°C . is sufficient to complete the fusion.

When fusion is complete, allow the crucible and contents to cool below 100°C ., then add 2 or 3 ml of water and heat over a low flame, using care to prevent spattering, until the contents can be separated from the crucible. Transfer the fused mixture to a 150 ml beaker with the aid of about 25 ml of hot water. Boil until the caked material is completely disintegrated, then filter thru a retentive filter paper. Wash the residue on the filter with two 15 ml portions of hot 5% Na_2CO_3 soln. Lead will be in both filtrate and residue. Transfer the filtrate to a separatory funnel and proceed to extract the lead from the filtrate as directed under aluminum lakes. Dissolve the residue on the filter in 10-20 ml of the hydrochloric acid soln, wash the filter with water and add washings to the soln. Boil the soln to expel carbon dioxide, then transfer to a separatory funnel and extract the lead as directed above. Combine with the chloroform extracts from the soluble portion of the fusion products and determine the total lead by the electrolytic method (29.39, 29.40, 29.41).

(5) The tentative method for pure coal-tar dye, 24.41 (p. 291), was adopted as official, first action, for pure dye in lakes of D&C Reds Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 31, and 34, and Ext. D&C Red No. 2.

22. DAIRY PRODUCTS

(1) The statement in the method for fat in cheese, 22.130 (p. 337), beginning "Add ca 0.5 g sand" lines 4 and 5, was changed to read "Add a few glass beads or other inert material."

(2) The official sour serum method 22.29 (p. 312) was deleted, first action.

(3) The following method was adopted as official, first action.

WATER-INSOLUBLE ACIDS IN BUTTER AND CREAM

Sampling of butter.—Proceed as directed in *Methods of Analysis*, A.O.A.C., 6th Ed., 22.107.

Preparation of butter sample.—Proceed as directed in *Methods of Analysis*, 6th Ed., 22.108 or 22.109. Weigh 50 g of the prepared sample into a 250 ml centrifuge bottle, add 10 ml of water, if necessary remelt in warm water bath (not steam bath), and add 50 ml of ether. Shake until fat is dissolved.

Preparation of cream sample.—Proceed as directed in *Methods of Analysis*, A.O.A.C., 6th Ed., 22.60. Weigh 50 g of the prepared sample into a 250 ml centrifuge bottle, add 20 ml of alcohol, shake, and add 50 ml of ether.

DETERMINATION

Add *N* NaOH in increments of about 0.2 ml to the material in the centrifuge bottle, neutralizing to a decided pink color, using 10 drops of a 1 per cent solution of phenolphthalein as indicator, shaking between additions of the alkali until neutralization is complete. Then add 0.5 ml in excess and shake again for at least 2 minutes. During this and all subsequent shakings release the pressure carefully several times to avoid blowing the stopper out and losing some of the contents. (It is difficult to shake more than one bottle at a time because of greasy stoppers and the pressure which develops.) After shaking remove the stopper and add 50 ml of petroleum ether, shake a few times and centrifuge for 5 minutes at about 1200 r.p.m. (longer if separation not sharp). Set the bottle on a horizontal surface and siphon¹ off the ether fat layer.² Wash the aqueous layer remaining in the centrifuge bottle by adding 25 ml of ether, mix thoroly by shaking for several seconds, add 25 ml of petroleum ether and again mix by shaking. Centrifuge, siphon off the ether layer as before, and repeat the washing as above. After each washing the basic red color of phenolphthalein should be permanent; if not, add additional phenolphthalein and alkali to give a decided red (not pink). Add 1 ml of sulfuric acid (1+1) to the residue in the centrifuge bottle and shake vigorously for a few seconds. With butter, add 5 ml and with cream, 10 ml of a 10 per cent sodium tungstate solution and again shake vigorously a few seconds. After the addition of the sodium tungstate, the material should be distinctly acid to congo red paper; if not, add more of the sulfuric acid. Now add 75 ml of ether, shake violently for at least 2 min. and centrifuge. (When working with cream, emulsions may form which do not break completely on centrifuging. These can be broken by adding 10 to 20 ml of alcohol, mixing gently and again centrifuging.) Siphon off the ether layer into a 500 ml separatory funnel. Wash the siphon inside and out with 75 ml of ether in such a manner that the washings drain into the centrifuge bottle, shake violently for at least 2 min., centrifuge and siphon off the ether layer into the separatory funnel. Slight opalescence of the ether layer may be disregarded. Add 100 ml of dilute alcohol (1+1) to the combined extracts in the separatory funnel, neutralize in same manner as before with *N* NaOH to a decided pink color, add 0.5 ml excess and shake violently for an additional 2 min. Immediately add 25 ml of water, mix by single inversion of the funnel, and allow to separate until the water layer is clear. This usually occurs in a few min. Slow separation may sometimes be hastened by playing a fine stream of water on the ether surface. If the volume of the emulsion at the interface is only about 10 ml it may be included in the subsequent extraction. Draw off the aqueous layer into a 600 ml beaker. Add 50 ml of the 1+1 alcohol and about 10 drops of phenolphthalein to the

¹ The siphon is similar to the delivery tube of an ordinary wash bottle except the intake end is bent, in opposite direction to the outlet end, into a U shape, the opening being $\frac{1}{4}$ to $\frac{1}{2}$ inch higher than the bottom of the U and cut off horizontally. Excessive constriction should be avoided in the bending. The delivery tube is set loosely enough in the stopper so that it can be raised and lowered. In operation it is so adjusted that the opening of the U bend is about $\frac{1}{4}$ inch above the surface of the aqueous layer. The ether layer can then be blown off gently by means of the customary mouthpiece tube inserted in an adjacent hole in the stopper.

² If the ether layer, after centrifuging, is reddish in color, add 10 ml of water, shake and again centrifuge as before. If the reddish color still persists in the ether layer, add 25 ml of ethyl ether, shake and again centrifuge.

contents of the separatory funnel and neutralize with the alkali, shaking vigorously for about 2 min. Add 50 ml of water, mix by single inversion of the funnel and allow to separate until the water layer is clear. Draw off the aqueous layer into the beaker. Add 10 ml of water to the contents of the separatory funnel, mix by single inversion, allow to separate until the water layer is clear, and draw off into the beaker. Place the beaker containing the combined extract and washings on a steam bath (or carefully heat on a hot plate), in order to expel any ether. Evaporate to about 25 ml (a small fan is useful if foaming is serious). (The decided red color should persist thru all these operations and up to the point where the soaps are acidified.) Transfer to a 250 ml beaker with about 25 ml of water. (As an alternate procedure the material may be evaporated to dryness on a steam bath and the residue dissolved in about 50 ml of water.) Dissolve 5 g of anhydrous sodium sulfate in the warm solution, using heat if necessary. Cool to 20° or lower, stirring at frequent intervals in order to keep the soaps from forming a hard crust on the surface. Make acid by adding sulfuric acid (1+1) dropwise, using congo red paper as indicator. Stir vigorously to affect thoro liberation of the fatty acids, mashing all pink soap curds. Add about 500 mg of a filter aid and mix. Filter with suction into a suitable filter.³ Rinse the beaker with 3 approximately 15 ml portions of water at 20° and transfer the rinsings to the crucible. Maintain suction for *several minutes after visible dripping has ceased*, in order to dry the precipitate. Heavy precipitates can be sucked drier if the cracks are plastered up with some of the precipitate. Filtrate should be clear. Substitute a tared beaker⁴ or flask, containing a few glass beads or grains of sand, for the receiving flask of the filtering apparatus. Extract the acids with 4 portions (ca 15 ml each) of ether, breaking up the precipitate with a stirring rod between extractions and thoroly mixing with the ether. The asbestos pad must not be disturbed. Evaporate the ether extract (which should be no more than faintly opalescent) on a steam bath and dry the acids in a 100°C oven for one hour. Cool and weigh. Report results as mg of water-insoluble acids (WIA) per 100 g of butterfat.

Dissolve the weighed acids in 10 ml of neutral benzene, and titrate with 0.1 N sodium ethylate, using 10 drops of phenolphthalein as indicator, until the end point holds at least 1 minute. (If desired, neutral alcohol and 0.1 N NaOH may be used.) Compute the mean molecular weight of the fatty acids by dividing the mg of acids found by the ml of 0.1 N alkali used for the titration, and multiplying by 10. The mean molecular weight should not exceed 290. When the amount of acids is below 150 mg per 100 g of butterfat, the mean molecular weight is without significance.

23. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

24. FISH AND OTHER MARINE PRODUCTS

(1) The following method was adopted as official, first action.

CRUDE FAT-ACID HYDROLYSIS

PREPARATION OF SAMPLE

Prepare the sample, according to the type of pack, as directed under par. 24.2 (*Methods of Analysis*, A.O.A.C., 1945, 359) and keep ground material in a sealed jar. If the jar has been chilled, allow the sample to come to room temperature and shake

³ The following set-up is convenient: a bell jar; a Gooch crucible, with removable bottom charged with a thin layer of asbestos overlaid with a small quantity of filter aid. The asbestos was a long fiber, amphibole variety, acid and alkali-washed for Gooch crucibles, and washed twice by decantation. The filter aid was Dicalcite Company's "Speedex" added from a suspension in water. Coarse fitted glass crucibles overlaid with a small quantity of filter aid are satisfactory.

⁴ Weighed with similar vessel as counterpoise.

jar so that any separated liquid will be absorbed by the fish. Open jar and stir contents with spatula, thoroly contacting the sides and lid so as to incorporate any separated liquid or fat.

METHOD

Weigh into a 50 ml beaker 8 grams of the well mixed sample. Add 2 ml of HCl. Break up coagulated lumps with a stirring rod having an extra large flattened end and continue until a homogeneous mixture is obtained. Add an additional 6 ml of HCl, mix, cover with watch-glass and heat on the steam bath for 90 min. with an occasional stirring. Cool solution and transfer to Mojonnier fat extraction tube. Rinse beaker with 7 ml of ethanol, add to extraction tube and mix. Rinse beaker with 25 ml of ethyl ether in three portions and add to extraction tube, stopper with cork or stopper of synthetic rubber unaffected by usual fat solvents, and shake tube vigorously for one min. Add 25 ml of petroleum benzine (b.p. below 60°) to extraction tube and repeat vigorous shaking. Centrifuge Mojonnier flask 20 min. at ca 600 r.p.m. and proceed as directed under par. 20.16 (*Methods of Analysis*, A.O.A.C., 1945, 240) beginning "Draw off as much as possible of ether-fat soln."

Drying to constant weight takes ca 40 min. for fish. Long heating periods tend to increase the weight of the fat. If a centrifuge is not available, the extraction can generally be made by letting Mojonnier flasks stand until the upper liquid is practically clear, then swirling flask and again letting stand until clear. If a troublesome emulsion occurs, draw off as much of the ether-fat soln as possible after letting flask stand, add a ml or two of ethanol, swirl, and again allow mixture to separate.

25. FLAVORING EXTRACTS

No additions, deletions, or other changes.

26. FRUITS AND FRUIT PRODUCTS

(1) In the official gravimetric cobaltinitrite method for potassium the following was inserted in 26.18(a) (p. 386) between "10 ml" and "Filter" line 2, "from a previously tested lot of the reagent giving recovery of not less than 98 and not more than 102% with 20 mg amounts of K_2O ."

(2) In note (3) of 26.19 (p. 387) the last sentence beginning "If recoveries" was deleted.

(3) The following method was adopted as official, first action.

WATER-INSOLUBLE SOLIDS (RAPID METHOD)

APPARATUS

Waring Blendor (or other suitable comminuting device).

Balance (sensitive to ± 1 milligram) and weights.

1-liter suction flask (provision for vacuum).

Büchner funnel (Coors #4 5½" diam.).

Filter paper.—15.0 cm fast; open texture (Whatman #4, or equivalent).

Weighing dishes.—Aluminum or tinned iron 5½" diam. $\times \frac{1}{4}$ " high, with close-fitting cover (16 mm film holders obtainable from camera stores). (All dishes weigh approximately 40 g, tinned iron ca 85–90 g.)

Rapid drying device:

(a) Moisture Teller Model 271 T, manufactured by Harry W. Dietert Co., 9330 Roselawn Ave., Detroit 4, Mich., or

(b) Forced Draft Drying Oven operating at 100°C.

DETERMINATION

Fit a 15 cm circle of filter paper into a 12½ cm Büchner funnel, add ½ of a 7 cm circle of filter paper (to be used to wipe any insoluble solids from Büchner after filtration and washing the sample), wash with boiling water, apply suction, and dry, using moisture teller and pan. Transfer to weighing dish, cool and weigh in balance using a tare consisting of weighing dish and paper. (Approximate time of drying, 5 min. at 215°F. \pm 5°F.)

Weigh 25 or 50 g of well mixed sample (Waring Blendor) to nearest .01 g, transfer the sample with hot water to a 400 ml beaker, adjust to approximately 200 ml with hot water, stir, and boil gently for a few minutes. Place prepared filter in Büchner, attach to suction flask but do not attach flask to suction line. Pour 50 to 100 ml of boiling water on filter and when a steady flow of water passes thru filter, transfer the sample to the filter, portionwise if necessary. Wash insoluble solids with boiling water and collect approximately 850 to 900 ml of filtrate. (In the washing operation keep the solids from forming a tight mat on the surface by portion-wise additions of the boiling water.) Apply suction after concluding the washing operation and aspirate thoroly. Transfer paper and water-insoluble solids to moisture teller pan, using extra piece of weighed filter paper to complete the transfer, and dry at 215°F. \pm 5°F. (approximately 15 min., depending on amount of water-insoluble solids). After drying, transfer sample to weighing dish, cool in desiccator, and weigh. Weight of water-insoluble solids + wt. of sample \times 100 = % water-insoluble solids.

(4) The following method was adopted as official, first action.

SEEDS IN BERRY FRUITS

Prepare the sample by thoro mixing, using a Waring Blendor. Take 50 g \pm .01 g of the sample, transfer with ca 500 ml of hot water to the mixing chamber of Waring Blendor and mix for 1-2 min. Transfer mixture to a 20-mesh screen and use additional hot water to transfer and wash the bare seeds. (Hot water from the tap is suitable for use in this procedure.) Transfer the seeds on the screen to a 70 mm aluminum dish, previously weighed, with close-fitting cover. (This is readily accomplished by transfer to a 7 cm Whatman #4 circle of filter paper in a Coors 2A Büchner funnel with suction. The paper is previously dried and weighed with the aluminum dish.) Dry at 100°C. in a forced draft oven for 30 min. and weigh. To determine average weight of one seed, count out and weigh separately several 100-unit lots. Report average weight of one seed in milligrams; number of seeds per 100 g of sample and after determination of the water-insoluble solids content of the sample, calculate and report the per cent of the total that is due to bare seeds and the per cent that is due to non-seed water-insoluble solids.

27. GRAIN AND STOCK FEEDS

(1) The tentative methods for calcium and phosphorus, *This Journal*, 31, 98 (1948), was adopted as official, first action.

(2) The tentative method for fat in fish meal, *This Journal*, 31, 98 (1948), was adopted as official, first action.

28. MEATS AND MEAT PRODUCTS

No additions, deletions, or other changes.

29. METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

No additions, deletions, or other changes.

30. NUTS AND NUT PRODUCTS

(1) The following methods were adopted as tentative.

Preservation of samples.—Preserve samples in glass top fruit jar or similar air-tight container at 5 to 10°C.

Preparation of sample:

(a) *Nuts in shell.*—Remove meats from shells, being careful to remove all particles of shell from meats. Prepare separated meats as in following.

(b) *Nut meats, shredded coconut, or small pieces.*—Grind not less than 250 g twice thru Enterprise No. 5 food chopper, equipped with revolving knife blade, and plate with holes about $\frac{1}{8}$ " diam. Other types of food choppers, graters, or comminuting devices that give a smooth homogeneous paste without loss of oil may be used. Mix sample well and store in air-tight glass container.

(c) *Nut butters and pastes.*—Transfer sample to container of convenient size and shape, warming semisolid products, and mix carefully with stiff bladed spatula or knife. Electric powered mixers or stirrers may be used instead of spatula or knife if the material is of the right consistency to give uniform mixture. Store sample in air-tight glass container.

Moisture:

Drying with heat.—Dry to constant weight at 95–100° under pressure not to exceed 100 mm of Hg (ca 5 hours), a quantity of the substance representing ca 2 g of dry material. Report loss of weight as moisture.

Crude Fat.

(a) *Direct method.*

Determination.—Large quantities of soluble carbohydrates may interfere with complete extraction of the fat. In such cases extract with H₂O before proceeding with the determination. Extract ca 2 g of sample, dried as directed under anhydrous ether (27.24) for 16 hours. Dry extract at temp. of boiling H₂O for 30 min., cool in desiccator, and weigh; continue at 30 min. intervals this alternate drying and weighing until weight is constant. One to 1.5 hours is usually required.

(b) *Indirect method.*—Determine moisture as directed under "Moisture"; then extract dried substance for 16 hours as directed under (a) above, and dry again. Report loss in weight as ether extract.

Crude protein.—Determine *N* as directed under 2.24, 2.25, or 2.26 (if over 30% protein use 2.26 only), and multiply result by 6.25.

Crude fiber.—See 27.28, 27.29, and 27.30.

Ash.—See 34.9 or 34.10 if chlorides present.

Reducing sugars.—See 27.31.

Sucrose.—See 27.32.

Sodium chloride:

(a) *Open Carius method.*—Put 2 g of the prepared sample into 250 Erlenmeyer flask or beaker, and proceed as in 24.6, line 3, beginning "Add."

(b) *With calcium acetate as fixative.*—To 2 g of prepared sample add and thoroly incorporate 10 ml of 10% acetate soln. Then begin with 3rd sentence of 24.7.

Water-insoluble inorganic residue. See 42.24.

Added coloring matters. See Chapter 21.

Metals, other elements, and residues. See Chapter 29.

Preservatives and artificial sweeteners. See Chapter 32.

(3) The following tentative methods were deleted: 30.1–30.10, inclusive, and 30.13–30.17, inclusive.

31. OILS, FATS, AND WAXES

(1) The modified tentative Bellier Test, 31.47–31.48, (p. 508), was modified as follows and adopted as official, first action.

31.48 Line 1, after "0.92 g" add "or measure 1 ml."

Line 2, end the first sentence with word "joint"; introduce between "joint" and "add" the new sentence, "If the oil is measured, use a short Mohr pipet with fairly large opening at top, drain to lower mark, hold until meniscus stops rising in pipet, and drain to mark again."

32. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) The official, first action, method for quarternary ammonium compounds in commercial preservatives, *This Journal*, 31, 105 (1948), table sirup, *ibid*, 31, 108, bottled beverages containing fruit juices, *ibid.*, 31, 106, and in beer, *ibid.*, 31, 108, were adopted as official, final action.

(2) To the official, first action, method for monochloroacetic acid, *This Journal*, 31, 104 (1948), the following was added and the method adopted as official, final action.

The following equally efficient means of extraction may be used: To 100 ml of sample add 3 ml of H_2SO_4 and shake in a separatory funnel with three equal volumes of ether. Unite the ether extracts and wash by shaking with two 30 ml portions of 1 *N* NaOH. Unite the two NaOH solutions and digest as above.

(3) The official method for monochloroacetic acid (above, No. 2) was adopted as official, first action, for carbonated beverages, and for beer and wine, with the addition "(Use the length of time found necessary for recovery of at least 95% when known quantities of monochloroacetic are extracted in the apparatus)" after "2–3 hours" line 4 of "determination."

(4) The following method was adopted as official, first action.

DETERMINATION OF MONOCHLOROACETIC ACID IN COMMERCIAL PRESERVATIVES

Prepare a dilution of the sample that will permit the measurement of a convenient aliquot containing 50–100 mg of monochloroacetic acid and determine in such aliquot as directed under the method for carbonated beverages.

(5) The following qualitative test for monochloroacetic acid in commercial preservatives was adopted as official, first action.

(A) BARIUM TEST

Dilute 4–5 ml of sample to 100 ml, add 6 ml of H_2SO_4 (1+1), and extract with an equal volume of ether in separatory funnel. In cases where emulsions form, extract in a continuous extractor for 1 hour. Transfer the ether extract to a separatory funnel, add a few drops of phenolphthalein indicator, 5 ml of ± 0.1 *N* $Ba(OH)_2$, and shake for 30 seconds. If the water layer takes on the pink color of phenolphthalein, transfer thru a filter paper to a small beaker. Add ± 0.05 *N* acetic acid until colorless and evaporate to 1–2 ml on the steam bath. Allow the remaining liquid to evaporate

spontaneously in the air and finally in a desiccator. If 5 ml of $\text{Ba}(\text{OH})_2$ does not give a pink water layer, add 5 ml more before separating. Repeat the extraction with $\text{Ba}(\text{OH})_2$ several times or until a pink soln is obtained, evaporating each barium soln in a separate beaker. Examine the crystals under the polarizing microscope.

Optical-Crystallographic Properties of Barium Monochloracetate

Barium monochloracetate monohydrate crystallizes from water in plates, many of which are hexagonal in habit and frequently forming in over-lapping layers. Even in material that has been finely powdered for microscopic examination, the pointed terminations of the plates, often in pairs, can be observed. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative on the more elongated plates. The plates invariably extinguish sharply with crossed nicols and therefore interference figures were not observed in convergent polarized light (crossed nicols). In view of the fact that the plates persistently lie in one orientation the significant refractive indices were determined by the statistical method, measuring the lowest and highest indices respectively on plates showing the maximum amount of double refraction. These two indices are therefore arbitrarily designated as n_α (the minimum value) and n_γ (the maximum value). The two significant refractive indices are: $n_\alpha = 1.582$ and $n_\gamma = 1.611$, both ± 0.002 , and frequently shown on the platy fragments.

(B) BARIUM-INDIGO TEST

REAGENTS

Anthranilic acid reagent.—Dissolve 1 g anthranilic acid in 40 ml of H_2O , add 0.3 g NaOH , and make up to 50 ml.

Caustic soda soln.—Dissolve 10 g NaOH in 10 ml H_2O and filter if necessary.

PROCEDURE

Dissolve 0.17 g of the barium salt in 5 ml H_2O in a 10 ml graduate, add 1.05 ml of 1.0 N H_2SO_4 , make up to 10 ml, and mix. Let stand until the precipitate settles or filter if preferred. Pipet 3 ml of the clear liquid into small beaker, add 2 ml anthranilic acid reagent and 30 mg Na_2CO_3 (weighed). Test with litmus paper. If acid, add one additional 30 mg of Na_2CO_3 . Pour into test tube and heat in water bath for $\frac{1}{2}$ hr. Place the test tube in an oven at $125^\circ \pm 5^\circ\text{C}$. until only a moist residue remains. Remove from the oven, add 2 drops of caustic soda soln directly upon the residue. (If the residue is entirely dry, add 1–2 drops H_2O and let stand until absorbed by the residue before adding the strong NaOH), return to the oven until completely dry (at least 1 hour). Remove from the oven and heat the test tube at $310^\circ\text{--}320^\circ\text{C}^*$ until the contents assume an orange color. (This requires 15 seconds to 2 min., but must be carefully watched to remove from the heat as soon as the reaction is complete.) Cool slightly, add 5–7 ml H_2O from a wash bottle, splashing the water to incorporate air into it. Warm over a flame and blow air thru the soln 1–2 min. using a pipet or glass tube. Heat to boiling over the flame and again blow air thru the soln. (As the oxidation progresses, the soln turns red if monochloracetic acid is present, then green or blue or a combination of the two, and finally solid particles of indigo separate out. They have a tendency to rise to the surface at first.) Let the mixture stand about 10 min., then acidify slightly with HCl (1+1). After standing further for $\frac{1}{2}$ hr. filter and wash the precipitated indigo with water to remove acid. Allow the paper to dry in the air and preserve as an exhibit.

* For the fusion at $310\text{--}320^\circ\text{C}$ use a brass block having a well to contain the test tube and a second well to contain a thermometer. The block is wrapped with a coil of nicrome wire and the heat controlled by a variable voltage transformer. Analysts have used muffle furnaces, micro-burners, Wood's metal, or solder baths, etc., for the fusion with equal success.

(C) INDIGO TEST

Dilute 2 ml of sample to 100 ml, add 3 ml of H_2SO_4 and shake with 100 ml of ether. Add 3 ml of anthranilic acid reagent to the ether extract, evaporate at a low temp., filter off any insoluble matter and apply the indigo test as under (B) beginning "Test with litmus paper, etc."

(D) PYRIDINE TEST

Extract 2 ml of sample as under (C). Transfer the ether extract to a separatory funnel, add a small piece of a universal indicator paper and a sufficient amount of a satd soln of sodium bicarbonate (5 ml are usually sufficient) to the ether extract to make the aqueous layer alkaline (pH 7-8) after vigorous shaking. Add enough water to make the total volume of the aqueous layer ca 10 ml and shake again. Draw off the aqueous layer into a small separatory funnel and wash the ether with two successive 5 ml portions of water, also drawing off these wash solns into the small separatory funnel. Wash the combined extracts once with 5-10 ml of ether. Add ca 1 ml of sulfuric acid (1+1) in excess of the amount required to neutralize the alkaline soln (1.5 ml are usually sufficient) and extract the acidified soln with two 25 ml portions of ether. Wash the combined ether extracts once with 1 or 2 ml of water and allow the ether soln to stand a few minutes after drawing off most of the water and swirling to get as complete separation of water from ether as possible. Pour the ether thru a folded filter paper into a 200 ml flask. Wash the separatory funnel and filter paper with two 10-ml portions of ether.

To the ether filtrate add 0.5 ml of pyridine, a small glass bead, mix, and place on a steam bath to remove the ether. Evaporate to a volume of 2 or 3 ml and transfer immediately by means of an eye dropper pipet to a 15 ml centrifuge tube, washing the flask successively with 2, 1, and 1 ml portions of ether. Using the evaporation apparatus, reduce the volume of liquid in the tube to ca 0.3 ml.

Add enough pyridine to increase the volume to ca 0.5 ml (mark on tube is helpful) and place in a constant temperature bath at $60 \pm 2^\circ C$.

If crystals appear during the heating period the test is positive, and the test need not be continued. If crystals do not appear remove test tube from bath. Remove the excess pyridine by evaporation under reduced pressure using the evaporating apparatus. Placing the tube in a beaker of hot water facilitates the operation. When all of the liquid has been removed, add 0.5 ml of pyridine, mix well, centrifuge, and decant the supernatant liquid. Add ca 5 ml of ether, shake well, centrifuge, and decant. Add 1-3 ml of absolute alcohol (95% alcohol gives results 1-2% lower), depending upon the amount of precipitate, place the tube in a holder, and heat in a hot water or steam bath until the precipitate is dissolved, being careful to swirl the tube gently to avoid superheating and to boil the alcohol so slowly that no loss occurs. Cool in an ice bath, add ca 10 ml of ether, mix well, and allow to stand in the ice bath for about 5 min. Centrifuge, pour off the supernatant liquid, and wash the precipitate once with ca 5 ml of ether. If the tube now contains crystals of pyridine betaine the test is positive.

(6) The following qualitative tests for monochloroacetic acid were adopted as official, first action.

**MONOCHLOROACETIC ACID IN CARBONATED BEVERAGES,
ORANGE JUICE, AND WINE****QUALITATIVE TEST**

Preparation of sample.—Acidify two 100 portions of sample with 3 ml of H_2SO_4 and extract, using either continuous extractors or separatory funnels (state which is used) and apply the indigo test to one extract and the pyridine test to the other.

INDIGO TEST

Add 3 ml of anthranilic acid reagent to the ether extract and evaporate at a low temp. If any insoluble matter (oily or solid) separates out, filter the remaining liquid thru a small wet filter paper into a 50 ml beaker. If no insolubles come out, transfer the residue to beaker, add 30 mg Na_2O_2 and proceed as directed under Barium-indigo test procedure (b) above, beginning "Test with litmus paper," etc.

PYRIDINE TEST

Proceed as under Pyridine test (d) above beginning "Transfer the ether extract."

(7) The tentative method for thiourea in orange juice, *This Journal*, 31, 100 (1948) was deleted and the following adopted as tentative.

THIOUREA IN ORANGES AND ORANGE JUICE

Rapid Oxidation Method

REAGENTS

(a) *Modified Grote*.—Dissolve 0.5 g of sodium nitroprusside in 10 ml of H_2O in 50 ml Erlenmeyer flask. Weigh out 0.5 g of hydroxylamine hydrochloride ($\text{NH}_2\text{OH} \cdot \text{HCl}$) and 1 g of NaHCO_3 . Mix the two solids quite uniformly in a small beaker or porcelain dish by gentle grinding with a small pestle or flattened glass rod, crushing any lumps in the material. Brush off the rod or pestle and transfer the mixed solid totally to the nitroprusside soln with the aid of a short-stemmed funnel and brush. Do not agitate the flask and allow it to stand until the evolution of CO_2 subsides to only a small evolution. Then swirl to dissolve any remaining NaHCO_3 . When the evolution of CO_2 practically ceases, add 0.10 ml (11 small drops) of bromine. A second evolution of gas occurs. When agitation no longer produces effervescence, make to 25 ml volume with distilled water and filter. Test the reagent for its efficacy as follows: Dilute 2 ml as in (b), add 1 ml diluted reagent to 10 ml soln composed of 5 ml reagent (c) (diluted 10 \times), 5 ml H_2O , and 1 drop acetic acid. A strong blue color should develop in 5 min. (If it does not, new reagent (a) should be prepared and the test repeated). Allow to stand at room temp. for 5–10 hours to age the soln. (The soln should be a mahogany brown color. If it is of a greenish cast, it is not as effective a reagent and soon loses its value.) Preserve this stock soln in the refrigerator and it will keep for several weeks.

(b) *Dilute Grote's reagent*.—Dilute one volume of the above reagent with four volumes of water before use and take 1 ml of the diluted reagent for a determination. The diluted reagent will keep for a day.

(c) *Thiourea stock soln*.—Dissolve 100 mg of the pure chemical in water and dilute to 200 ml.

(d) *Citric acid-potassium citrate soln*.—Dissolve 1 g of citric acid (reagent) and 0.84 g of potassium citrate ($\text{K}_3\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$ U.S.P.) in water, and make to 100 ml.

(e) *Iodine soln*.—Standard 0.1 N soln in KI.

(f) *Sulfuric acid*.—Ca normal soln (0.98 to 1.02 N).

APPARATUS

Siphon.—Insert two bent glass tubes in a two-hole cork or stopper, one terminating just below the stopper and the other long enough to reach the bottom of a centrifuge bottle when the cork with tubes is inserted in the mouth of the bottle. Attach another glass tube to the outside end of the longer bent tube by means of a flexible rubber tube. The assembly is used to siphon the lower layer from a centrifuge bottle, and the rate of flow is controlled by squeezing the rubber connection.

Prepare a cap for the inner tube by boring a hole of the same diam. as the tube part way thru a small cork.

PREPARATION OF SAMPLE

Juice oranges in an ordinary reamer, strain out seeds and pulp, and mix well. Measure 125 ml into a 250 ml centrifuge bottle, add 70 ml of ethyl ether, and shake well for 1–2 min. Centrifuge the bottle and contents about 10 min. at 1800 r.p.m. and remove. Cap the short end of the siphon, insert it into the bottle, and lower it thru the top layer into the lower aqueous layer. Push off the cork cap with a glass rod. Lower the tube to the bottom of the bottle, push cork (which carries the siphon) into the mouth of the bottle, and blow in the short tube to start the flow of liquid. Carefully siphon off into a beaker as much of the lower layer as possible and control the rate of flow by squeezing on the rubber connection. Stop the flow when material from the center emulsion layer begins to enter the tube. Add a teaspoonful of Celite (filtercel) to the siphoned liquid in the beaker, stir well, and filter on a Büchner funnel (7–11 cm) with suction, using a Whatman No. 54 or 41 H filter paper. Warm the flask and contents to ca 36°C. (on steam bath) and again apply suction to remove ether.

Pipet 25 ml of the filtrate (clear or nearly so) into a clean 50 ml volumetric flask. In two similar flasks place 25 ml aliquots of citric acid K citrate soln (reagent (d)). To one flask add 2 ml of standard thiourea soln (reagent (c)); the other is a blank. To each of the three flasks, add 5 ml of normal sulfuric acid. Then add 0.1 *N* iodine soln slowly, with rotation, to each flask until the iodine color does not disappear and add 1 ml in excess. Allow the flasks (samples, standard, and blank) to stand 10 min. at room temp. Now add a soln of NaHSO₃ (2.5 g/liter) to the contents of the flasks until the iodine color disappears and add 3 or 4 drops in excess. Add gradually and slowly with swirling, 4 ml of 25% sodium acetate soln to each flask and make to volume with water and mix. Designate the oxidized diluted sample as solution "X."

DETERMINATION

Prepare two standards by placing respectively 5 and 10 ml portions of soln from the standard flask in test tubes. Make the first tube to 10 ml by adding 5 ml of liquid from blank soln (no thiourea). Place 10 ml portions of the blank soln and of the sample soln ("X") in two other test tubes. Pipet 1 ml of diluted Grote reagent (b) into each tube with shaking or stirring. Allow the tubes to stand for one hour at about 25°C., or for 10–15 min. in a bath at 45–50°C., to develop the color (blue). Read the developed color of the solns from each tube (sample blank and standards) in a neutral wedge photometer using a filter centered at 610 m μ and a 1-inch photometer cell. From the readings of the blank and standards construct a curve (linear) plotting photometer readings against p.p.m. of thiourea. The oxidized standard in the flask represents 20 p.p.m. (1 mg in 50 ml). A 10 ml aliquot therefore represents 20 p.p.m. and 5 ml corresponds to 10 p.p.m.

A slight correction on the sample color reading obtained as above is necessary because of the natural color present in "X" before addition of the thiourea (Grote) reagent. Obtain readings on the blank soln and the sample soln ("X") as contained in the volumetric flask, without added reagent, using the same photo cell. Subtract the difference between these readings (X – blank) from the sample reading with the thiourea (Grote) reagent. Obtain from the graph the thiourea (p.p.m.) corresponding to the corrected reading. Multiply this value by 2 to obtain the thiourea concentration in the original orange juice.

33. SPICES AND OTHER CONDIMENTS

- (1) The official, first action, method for starch in mayonnaise and

salad dressing, *This Journal*, 31, 108 (1948), was adopted as official, final action.

(2) The official, first action, method for starch in prepared mustard and mustard flour, *This Journal*, 30, 75 (1947) as revised, *This Journal*, 31, 108 (1948), was adopted as official, final action.

(3) The tentative method for permanganate oxidation number of vinegar, 33.91-33.92 (p. 554) was deleted and the following adopted as official, first action.

PERMANGANATE OXIDATION NUMBER

REAGENTS

(a) *Sulfuric acid soln.*—1+1.

(b) *Potassium permanganate soln.*—31 g per 1000 ml. Standardization is not necessary. Prepare soln according to 43.17, p. 807.

(c) *Sodium thiosulfate*—0.5 N.—Accurately standardized against $K_2Cr_2O_7$. (43.28, p. 809) should be modified to correspond to the stronger $Na_2S_2O_3$. It has been found that the following quantities of reagents are satisfactory: 0.50 g $K_2Cr_2O_7$, 10 g KI, 10 ml conc. HCl, and 90 ml H_2O .

(d) *Potassium iodide soln.*—Dissolve 50 g of KI in 100 ml H_2O and filter. Do not use unless colorless.

DETERMINATION

Adjust vinegar to 4 g/100 ml acidity as acetic acid. Steam distil 50 ml of adjusted vinegar and collect 50 ml of distillate. (Distillation should be regulated so that ca 45 ml remain in distilling flask when 50 ml of distillate have been collected. All-glass apparatus is preferable; if not available, cork or rubber stoppers should be covered with Sn or Al foil. The apparatus used for determination of volatile fatty acids in fish products (24.9, p. 361) is very convenient. Keep distillate and reagents at 25°C.)

Transfer the 50 ml of distillate to 500 ml glass-stoppered Erlenmeyer flask. Add 10 ml of the H_2SO_4 soln and 25 ml of the $KMnO_4$ soln. The permanganate should be accurately measured, allowing the pipet to drain for a definite time. Hold at 25°C, preferably in a water bath, for exactly one hour. Then immediately add 20 ml of the KI soln and mix well. Titrate the liberated I with the 0.5 N $Na_2S_2O_3$.

Conduct a blank determination at the same time, using 50 ml of H_2O , 10 ml of the H_2SO_4 soln, and 25 ml of the $KMnO_4$ soln.

(4) The tentative method for gums in mayonnaise and French dressing, 33.57 (p. 548), was changed to substitute "50 ml" in line 3 of paragraph 2 for "1.5 oz.") and adopted as official, first action.

34. SUGARS AND SUGAR PRODUCTS

(1) The following method was adopted as official, first action.

LAC IN CANDIES

Place 50 g of candy in a 400 ml beaker. Add 50 ml of a mixture of benzol and absolute alcohol (50% by volume) and cover with a watch-glass. Place on the steam bath, heat to boiling, and simmer for a few minutes, stirring occasionally. Decant the liquid into a tared round glass dish of about 100 ml capacity, having a flat bottom about 2½" in diam. Repeat, using a similar mixture; and finally rinse twice with

two 25 ml portions of absolute alcohol, simmering and stirring each rinsing liquid. With moist sugar candy, avoid overheating to prevent pieces from sticking together.

Add each liquid to the glass dish previously placed over the steam to evaporate the alcohol-benzol mixture. Allow to remain on bath until alcohol is just removed, rotating the dish as it goes to dryness in order to spread the extract uniformly over the bottom surface. Avoid baking the shellac on the dish. If fat appears to be present wash with 3 15-ml portions of petroleum benzene, stirring, and warming. Decant thru a rapid filter.

Add 50 ml of a mixture of 25 ml isoamyl alcohol (B.P. 129–132) and 25 ml benzol, rinsing any solid matter off and filter back into dish. Heat on steam bath with stirring, cool somewhat, and transfer the soln with suspended matter to a suitable (125 ml) separatory funnel. Rinse the dish with 25 ml hot (about 60°) water, adding it to the funnel; shake well, and filter wash water if necessary. Repeat washing *two times* (or until washings are colorless) with water, rinsing the dish well around sides with the first portions of the liquid. Finally, filter the soln of the shellac into the tared dish, rinsing the separator and filters with a little absolute alcohol. Evaporate to dryness on the steam bath, rotating the dish on going to dryness to give a uniform film.

If much fat was extracted in original benzol extraction, wash the final shellac residue with 25 ml petroleum ether, warming and stirring. Decant, dry on steam bath and 100° oven, and weigh.

(2) The tentative method for unfermented reducing substances in molasses, *This Journal*, 31, 109 (1948) was revised to change reagent (f) from 5 volumes of water to 3, and add after "time" line 6 under fermentation, "an incubator may be used and the flask left overnight," and adopted as official, first action.

(3) The tentative Ofner method for invert sugar in presence of sucrose. 34.47 and 34.48 (p. 575), was adopted as official, first action.

(4) The official refractometric method 34.8 (p. 558) was revised by addition of the following (first action): "In liquid products containing invert sugars, correct the per cent solids obtained from 44.7 by adding 0.022 for each per cent invert sugar present in the sample."

35. PROCESSED VEGETABLE PRODUCTS

No additions, deletions, or other changes.

36. VITAMINS

(1) The following changes were adopted in the tentative method for vitamin A in fish liver oils (pages 599–601) and the method adopted as official, first action:

36.2 Insert "U.S.P. ethyl" before the word "ether" and "anesthesia grade, free from peroxides" after the word "cans" in line 2.

36.3 Delete the word "ground" from end of line 2.

Delete "2 minutes" from line 7 and substitute "allow the mixture to stand (about 2 minutes) until separation is visibly complete, as determined by the absence of refraction streaming and the presence of distinct layers."

36.4 Change "0.4" line 4 to "0.398."

36.6 The density values in the table were corrected as follows:

T.	D.
0.7	2.16
7.0	1.16
11.0	0.959
26.5	0.577
27.5	0.561
29.5	0.530
32.5	0.488
55.5	0.256
63.0	0.201
71.5	0.146

(2) The tentative method for thiamine hydrochloride (vitamin B₁) 36.16–36.23 (p. 606) was adopted as official, first action.

(3) The tentative fermentation method for thiamine, 36.27–36.31 (p. 611–613), was adopted as official, first action.

(4) The 1st paragraph of the section on Preparation of assay solution, 36.25 (p. 610) of the fluorometric method for thiamine, official, first action, *This Journal*, 31, 112 (1948), was deleted and the following adopted as official, first action.

PREPARATION OF ASSAY SOLUTION

A. EXTRACTION STEP

(a) *For dry or semidry materials that contain no appreciable amount of basic substances.*

(1) Add a volume of 0.1 N HCl or 0.1 N H₂SO₄ equal in vol. to 10–15 times the weight of the sample in g. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occur agitate vigorously so that all particles come in contact with the liquid, then wash down sides of flask with acid soln.

(2) Digest at 95°–100°C. in a steam bath or in boiling water with frequent mixing, 30 min.; or alternatively autoclave the mixture at 121°–123° for 30 min. Cool and adjust pH to 4–4.5 by addition of the 2 N NaC₂H₃O₂ soln, using brom-cresol green pH indicator on spot plate.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances.*

Add HCl soln or H₂SO₄ soln and adjust the mixture to ca pH 6.0. Add such an amount of H₂O that the total volume of liquid is equal in ml to 10–15 times the dry wt of the sample in g. Then add the equivalent of 1 ml to 10 N HCl or 10 N H₂SO₄ for each 100 ml of liquid and proceed as directed under (a) (1).

(c) *For liquid materials.*

Adjust the material to ca pH 6.0 with either HCl soln, H₂SO₄ soln, or NaOH soln and proceed as directed under (b).

B. ENZYME HYDROLYSIS STEP

To a 75-ml aliquot add 5 ml of the enzyme soln, mix, and incubate at 45–50° for 3 hours. Cool, centrifuge mixture until supernatant liquid is clear or practically so, and quantitatively transfer supernatant liquid to a 100 ml volumetric flask. Wash residue by centrifuging with 10 ml, then with 5 ml of 0.1 N H₂SO₄. Add washings to the supernatant liquid and dilute to 100 ml with H₂O.

(5) In 36.26 (p. 610) line 8 “(2 seconds)” was changed to “(in 1–2 seconds).”

(6) The tentative method for riboflavin, *This Journal*, 30, 79 (1947), was deleted and the following adopted as official, first action.

RIBOFLAVIN (VITAMIN B₂)¹

Microbiological Method

Throughout all stages of the procedure, except where otherwise directed, protect the solns from light that destroys riboflavin.

REAGENTS

(a) *Standard riboflavin stock soln. I.*—Dissolve 50 mg of U.S.P. Riboflavin Reference Standard* in 0.02 *N* acetic acid soln to make 500 ml.¹ Store under toluene at ca 10°. 1.0 ml = 100 micrograms of riboflavin.

(b) *Standard riboflavin stock soln II.*—To 100 ml of (a), add 0.02 *N* acetic acid soln to make 1000 ml. Store under toluene at ca 10°. 1.0 ml = 10 micrograms of riboflavin.

(c) *Standard riboflavin soln.*—Dilute 5.0 ml of (b) with H₂O to make 1000 ml. 1.0 ml = 0.05 microgram of riboflavin. Prepare fresh standard soln for each assay.

(d) *Photolyzed peptone soln.*—Dissolve 40 g of peptone in 250 ml of H₂O, and 20 g of NaOH in 250 ml of H₂O, and mix the solns in a crystallizing dish having a diam. of ca 25 cm. At a distance of ca 30 cm from the dish, place a 100-watt bulb fitted with a reflector, and expose the soln to light from the bulb for 6–10 hours. Maintain the soln during this treatment at temp. not exceeding 25°. Neutralize the NaOH with acetic acid and add 7 g of anhydrous Na acetate and H₂O to make 800 ml. Store under toluene at ca 10°.

(e) *Cystine soln.*—Dissolve 1.0 g of L-cystine in 20 ml of HCl (1+3) and add H₂O to make 1000 ml. Store under toluene at ca 10°.

(f) *Yeast supplement soln.*—Dissolve 25 g of H₂O-soluble yeast extract in H₂O to make 125 ml, add 125 ml of a soln containing 38 g of Pb subacetate, and mix the solns. Filter, and add NH₄OH (1+2) to the filtrate to pH 10. Filter, and add acetic acid to the filtrate to pH 6.5. Precipitate the excess Pb with H₂S, filter, and add H₂O to the filtrate to make 250 ml. Store under toluene at ca 10°.

(g) *Salt soln A.*—Dissolve 25 g of KH₂PO₄ and 25 g of K₂HPO₄ in H₂O to make 500 ml. Add 5 drops of HCl and store under toluene.

(h) *Salt soln B.*—Dissolve 10 g of MgSO₄ · 7H₂O, 0.5 g of NaCl, 0.5 g of FeSO₄ · 7H₂O, and 0.5 g of MnSO₄ · H₂O in H₂O to make 500 ml. Add 5 drops of HCl and store under toluene.

(i) *Basal medium stock soln.*—

Photolyzed peptone soln	= 50 ml
Cystine soln	= 50 ml
Yeast supplement soln	= 5.0 ml
Dextrose, anhydrous	= 15 g
Salt soln A	= 5.0 ml
Salt soln B	= 5.0 ml

Dissolve the anhydrous dextrose in the solns previously mixed and adjust to pH 6.8 with NaOH soln. Finally add H₂O to make 250 ml.

(j) 10 *N* HCl soln.

(k) 1 *N* HCl soln.

(l) 0.1 *N* HCl soln.

(m) 10 *N* NaOH soln.

(n) 1 *N* NaOH soln.

(o) 0.1 *N* NaOH soln.

¹ See reference at end of method.

* U.S.P. Riboflavin Reference Standard may be obtained from the U.S.P. Reference Standards, 4738 Kingessing Ave., Philadelphia 43, Pa.

PREPARATION OF INOCULUM

(a) *Stock culture of Lactobacillus casei*.—Dissolve 2.0 g of H₂O-soluble yeast extract in 100 ml of H₂O, add 0.5 g of anhydrous dextrose, 0.5 g of anhydrous Na acetate, and 1.5 g of agar, and heat the mixture, with stirring, on a steam bath until the agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug the tubes with cotton, sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and allow to cool in an upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of *Lactobacillus casei*,* incubating for 16 to 24 hours at any selected temp. between 30° and 37° but held constant to within $\pm 0.5^\circ$, and finally store at ca 10°. Prepare a fresh stab of the stock culture every week, and do not use for inoculum if the culture is more than 1 week old.

(b) *Culture medium*.—To each of a series of test tubes containing 5 ml of the basal medium stock soln, add 5 ml of H₂O containing 0.5 microgram of riboflavin. Plug the tubes with cotton, sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and cool.

(c) *Inoculum*.—Make a transfer of cells from the stock culture of *Lactobacillus casei* to a sterile tube containing 10 ml of culture medium. Incubate this culture for 16–24 hours at any selected temp. between 30° and 37° but held constant to within $\pm 0.5^\circ$. The cell suspension so obtained is the inoculum.

PREPARATION OF SAMPLE SOLUTION

Throughout all stages of the procedure, keep the soln below pH 7.0 in order to protect riboflavin.†

Place a measured amount of sample in a flask of suitable size and proceed by one of the methods given below.

(a) *For dry or semidry materials that contain no appreciable amount of basic substances*.—Add a volume of 0.1 N HCl soln equal in ml to not less than 10 times the dry weight of the sample in g, but the resulting soln shall contain not more than 0.1 mg of riboflavin per ml. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occurs, agitate vigorously so that all particles come in contact with the liquid, then wash down sides of flask with 0.1 N HCl soln.

Heat the mixture in an autoclave at 121°–123° (1.1–1.2 kg per sq cm) for 30 min. and cool. If lumping occurs, agitate the mixture until particles are evenly dispersed. Adjust the mixture, with vigorous agitation, to pH 6.0 with NaOH soln,[‡] then add HCl soln immediately until no further precipitation occurs (usually ca pH 4.5, the isoelectric point of many of the proteins). Dilute the mixture to a measured volume that contains more than 0.05 microgram of riboflavin per ml and filter thru paper known not to adsorb riboflavin. In the case of a mixture that is difficult to filter, centrifuging and/or filtering thru sintered glass (using a suitable analytical filter-aid) may often be substituted for, or may precede, filtering thru paper. Take an aliquot of the clear filtrate and check for dissolved protein by adding, dropwise, first HCl soln and, if no precipitate forms, then, with vigorous agitation, NaOH soln, and proceed as follows:

(1) If no further precipitation occurs, add, with vigorous agitation, NaOH soln to pH 6.8, dilute the soln to a final measured volume that contains ca 0.05 microgram of riboflavin per ml, and if cloudiness occurs, filter again.

(2) If further precipitation occurs, adjust the soln again to the point of maximum

* Pure cultures of *Lactobacillus casei* may be obtained from the American Type Culture Collections, 1099 M Street, N.W., Washington 6, D. C., as number 7469.

† The concentrations of the HCl and NaOH solns used are not stated in each instance because these concentrations may be varied depending upon the amount of sample taken for assay, volume of sample soln, and buffering effect of sample.

precipitation, dilute to a volume that contains more than 0.05 microgram of riboflavin per ml, and then filter. Take an aliquot of the clear filtrate and proceed as directed under (1).

If the riboflavin content of the sample is too low so that these requirements cannot be met, then concentrate the clear filtrate obtained at ca pH 4.5 to a suitable volume with heat under reduced pressure. Filter if necessary and then proceed as outlined above.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances.*—Add dilute HCl and adjust the mixture to pH 6.0. Add such an amount of H₂O that the total volume of liquid shall be equal in ml to not less than 10 times the dry weight of the sample in g. Then add the equivalent of 1 ml of 10 N HCl soln for each 100 ml of liquid and proceed as directed under (a).

(c) *For liquid materials.*—Adjust the material to pH 6.0 with either HCl soln or, with vigorous agitation NaOH soln, and proceed as directed under (b).

DETERMINATION

Prepare standard riboflavin tubes as follows: To duplicate test tubes, add 0.0 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml, 4.0 ml, 4.5 ml, and 5.0 ml, respectively, of the standard riboflavin soln. To each of these tubes add 5.0 ml of basal medium stock soln and H₂O to make 10 ml.

Prepare tubes containing the sample to be assayed as follows: To duplicate test tubes add, respectively, 1.0 ml, 2.0 ml, 3.0 ml, and 4.0 ml of the sample soln. To each of these tubes add 5.0 ml of basal medium stock soln and H₂O to make 10 ml.

After mixing, close the tubes by plugging with cotton, or, covering with caps, and sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm).^{*} Cool, aseptically inoculate each tube with 1 drop of inoculum, and incubate for 72 hours at any selected temp. between 30° and 37° but held constant to within $\pm 0.5^\circ$. Contamination of the assay tubes with any foreign organism invalidates the assay.

Titrate the contents of each tube with 0.1 NaOH soln, using bromothymol blue as the indicator, or to pH 6.8 measured electrometrically.

Prepare a standard curve of the riboflavin standard titrations by plotting the average of the titration values expressed in ml of 0.1 N NaOH soln for each level of the riboflavin standard soln used, against micrograms of riboflavin contained in the respective tubes. From this standard curve, determine by interpolation the riboflavin content of the sample soln in each tube. Discard any values of more than 0.20 or less than 0.05 microgram of riboflavin in each tube. Calculate the riboflavin content in each ml of sample soln for each of the tubes. The riboflavin content of the sample is calculated from the average of the values obtained from not less than 6 of these tubes that do not vary by more than $\pm 10\%$ from the average. If the titration values of less than 6 of these tubes containing the sample soln are within the range of the titration values of the riboflavin standard tubes containing 0.05 to 0.20 microgram of riboflavin, the data are insufficient to permit calculation of riboflavin content of the sample. Titration values exceeding 1.0 ml for the tubes of the standard riboflavin soln series containing 0.0 ml of the soln indicate the presence of an excessive amount of riboflavin in the basal medium stock soln and invalidate the assay.

REFERENCES

- ¹ *This Journal*, 23, 346 (1940); 24, 413 (1941); 25, 459 (1942); 26, 81 (1943); 27, 540 (1944); 28, 560 (1945); 29, 25 (1946); 30, 79 (1947); 31, 701 (1948).

^{*} Overheating (oversterilizing) of the assay tubes may cause unsatisfactory results.

¹ See footnotes at end of method.

(7) The following method was adopted as official, first action.

RIBOFLAVIN (VITAMIN B₂)

Fluorometric Method

REAGENTS

(a) *Standard riboflavin stock soln I.*—Dissolve 50 mg of U.S.P. Riboflavin Reference Standard* in sufficient 0.02 *N* acetic acid soln to make 500 ml.¹ Store, protected from light, under toluene at ca 10°. 1 ml = 100 micrograms of riboflavin.

(b) *Standard riboflavin stock soln II.*—To 100 ml of (a), add 0.02 *N* acetic acid soln to make 1000 ml. Store, protected from light, under toluene at ca 10°. 1 ml = 10 micrograms of riboflavin.

(c) *Standard riboflavin soln.*—Dilute 10 ml of (b) with H₂O to make 100 ml. 1 ml = 1 microgram of riboflavin. Prepare fresh standard soln for each assay.

(d) 10 *N* HCl soln.

(e) 1 *N* HCl soln.

(f) 0.1 *N* HCl soln.

(g) 10 *N* NaOH soln.

(h) 1 *N* NaOH soln.

(i) 0.1 *N* NaOH soln.

(j) Acetic acid.

(k) 4% KMnO₄ soln.

(l) 3% H₂O₂ soln.

(m) Na₂S₂O₄ powder.

APPARATUS

Electronic photofluorometer.—Use a fluorometer that is suitable for accurately measuring fluorescence of solns containing riboflavin in concentrations of ca 0.1 to 0.2 microgram per ml.

PREPARATION OF SAMPLE SOLUTION

Throughout all stages of the procedure, protect the soln from light that destroys riboflavin and keep the pH of the soln below 7.0.

Place a measured amount of sample in a flask of suitable size and proceed by one of the methods given below.

(a) *For dry or semidry materials that contain no appreciable amount of basic substances.*—Add a volume of 0.1 *N* HCl soln equal in ml to not less than 10 times the dry weight of the sample in g, but the resulting soln shall contain not more than 0.1 mg of riboflavin per ml. Commminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occurs, agitate vigorously so that all particles come in contact with the liquid, then wash down sides of flask with 0.1 *N* HCl soln.

Heat the mixture in an autoclave at 121°–123° (1.1–1.2 kg per sq cm) for 30 min. and cool. If lumping occurs, agitate the mixture until particles are evenly dispersed. Adjust the mixture, with vigorous agitation, to pH 6.0 with NaOH soln² and add HCl soln immediately until no further precipitation occurs (usually ca pH 4.5, the isoelectric point of many of the proteins). Dilute the mixture to a measured volume that contains more than 0.1 microgram or riboflavin per ml and filter thru paper known not to adsorb riboflavin. In the case of a mixture that is difficult to filter, centrifuging and/or filtering thru sintered glass (using a suitable analytical filter-

* U.S.P. Riboflavin Reference Standard may be obtained from the U.S.P. Reference Standards, 4738 Kingsessing Ave., Philadelphia 43, Pa.

¹ To facilitate soln, 50 mg of U.S.P. Riboflavin Reference Standard may be added to ca 300 ml. of 0.02 *N* acetic acid soln and the mixture warmed on a steam bath with constant stirring until riboflavin is dissolved. Then cool and add sufficient 0.02 *N* acetic acid soln to make 500 ml.

² The concentrations of the HCl and the NaOH solns are not stated in each instance because these concentrations may be varied depending upon the amount of sample taken for assay, volume of sample soln, and buffering effect of sample.

aid) may often be substituted for, or may precede, filtering thru paper. Take an aliquot of the clear filtrate and check for dissolved protein by adding, dropwise, first HCl soln and, if no precipitate forms, then, with vigorous agitation, NaOH soln, and proceed as follows:

(1) If no further precipitation occurs, add, with vigorous agitation, NaOH soln to pH 6.8, dilute the soln to a final volume that contains ca 0.1 microgram of riboflavin per ml, and if cloudiness occurs, filter again.

(2) If further precipitation occurs, adjust the soln again to the point of maximum precipitation, dilute to a volume that contains more than 0.1 microgram of riboflavin per ml, and then filter. Take an aliquot of the clear filtrate and proceed as directed under (1).

If the riboflavin content of the sample is too low so that these requirements cannot be met, then concentrate the clear filtrate obtained at ca pH 4.5 to a suitable volume with heat under reduced pressure. Filter if necessary and then proceed as outlined above.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances.*—Add dilute HCl and adjust the mixture to pH 6.0. Add such an amount of H₂O that the total volume of liquid shall be equal in ml to not less than 10 times the dry weight of the sample in g. Then add the equivalent of 1 ml of 10 N HCl soln for each 100 ml of liquid and proceed as directed under (a).

(c) *For liquid materials.*—Adjust the material to pH 6 with either HCl soln or, with vigorous agitation, NaOH soln and proceed as directed under (b).

DETERMINATION

To each of 4 or more tubes¹ (or reaction vessels) add 10 ml of sample soln. To each of 2 or more of these tubes add 1 ml of the standard riboflavin soln and mix, and to each of 2 or more of the remaining tubes, add 1 ml of H₂O and mix. To each of the tubes add 1 ml of acetic acid, mix, add, with mixing, 0.5 ml of KMnO₄ soln,² and allow to stand for 2 min.³ Then to each of the tubes, add, with mixing, 0.5 ml of H₂O₂ soln, whereupon the permanganate color must be destroyed within 10 seconds.⁴ Shake tubes vigorously until excess O is expelled. If gas bubbles remain on sides of tubes after foaming has ceased, remove bubbles by rotating tubes slowly from end to end.

In a suitable fluorometer, measure the fluorescence of the sample soln containing 1 ml of added standard riboflavin soln and call this reading "A." Next, measure the fluorescence of the sample soln containing 1 ml of added H₂O and call this reading "B." Then add, with mixing, 20 mg of Na₂S₂O₄⁵ to reduce the riboflavin present, to 2 or more tubes, measure the fluorescence within 5 sec, and call this reading C.⁶ The riboflavin content may then be calculated as follows.⁹

¹ If the fluorometer is of a type that requires tubular cuvettes, all reactions may be carried out in a matched set of these cuvettes.

² The amount of KMnO₄ soln may be increased for sample solns that contain an excess of oxidizable material, but do not add more than 0.5 ml in excess of that required to complete the oxidation of foreign material.

³ A riboflavin recovery step is not included in this procedure as no destruction of riboflavin occurs until an elapse of time of ca 3 minutes.

⁴ With the precipitation procedure properly followed for preparation of sample soln, no precipitate forms during the above reactions and, therefore, filtration at this point is necessary.

⁵ The Na₂S₂O₄ must be of high purity. Do not use if unduly exposed to light or air. An amount appreciably in excess of 20 mg may reduce foreign pigments and/or foreign fluorescing substances thereby causing erroneous results. The suitability of the Na₂S₂O₄ is checked by the following manner:

To each of 2 or more tubes, add 10 ml of H₂O and 1 ml of standard riboflavin soln containing 20 micrograms of riboflavin per ml, and proceed as directed above with respect to the addition of acetic acid, KMnO₄ soln, and H₂O₂ soln. Then upon the addition, with mixing, of 8 mg of Na₂S₂O₄, the riboflavin is completely reduced in not more than 5 sec.

⁶ After reduction, tubes of A and B should give the same degree of fluorescence.

⁹ Most accurate results are obtained when the sample soln is of such a dilution that $B - C/A - B$ approaches 1/1. Any determination in which there is an appreciable deviation from this ratio should be considered a preliminary assay and should be repeated using a dilution of the sample soln that would give readings more nearly approaching this ratio.

$$\text{Mg of riboflavin per ml of final sample soln} = \frac{B-C}{A-B} \times \frac{1}{10} \times \frac{1}{1000}.$$

Calculate the amount of riboflavin in the sample on the basis of the aliquots taken during the analysis.

(8) The official, first action, method for vitamin C, 36.47-36.48, (p. 620), was revised as follows and adopted as official, final action.

In parenthetical expression under the title after "ferrous Fe" add "Stannous Sn" and "cuprous Cu."

Delete "freshly pulverized stick H_3PO_3 " in 36.47(a) and substitute "glacial HPO_3 pellets, or freshly pulverized stick."

Delete note at end of method (p. 621) and substitute the following:

Products containing ferrous Fe, stannous Sn, and cuprous Cu give values in excess of their actual ascorbic acid content by this method. Following are simple tests to ascertain whether these reducing ions are present in appreciable quantities to invalidate analysis: Add 2 drops of 0.05% H_2O soln of methylene blue to 10 ml of freshly prepared mixture of juice and the HPO_3 -acetic acid reagent, mix. Disappearance of methylene blue color in 5-10 seconds indicates presence of interfering substances. Stannous Sn does not give the test and may be tested for by using another 10 ml sample soln to which 10 ml of 25% HCl is added, mix, then 5 drops of 0.05% H_2O soln of indigo carmine, mix. Disappearance of indigo carmine color in 5-10 seconds also indicates presence of interfering substance.

(9) The tentative method for vitamin D in milk, 36.49-36.60 (p. 621), was adopted as official, first action.

(10) The alternate tentative method for carotene, *This Journal*, 31, 111 (1947), was adopted as official, first action, for carotene in hays and dried plants.

(11) The tentative method for carotene, *This Journal*, 30, 84 (1947), was deleted for hays and dried plants but continued as tentative for analysis of other materials.

(12) The official, first action, method for nicotinic acid, *This Journal*, 30, 82 (1947), was revised as follows:

NICOTINIC ACID (NIACIN) OR NICOTINAMIDE (NIACINAMIDE)¹

Microbiological Method

REAGENTS

(a) *Standard nicotinic acid stock soln I*.—Dissolve 50 mg of U.S.P. Nicotinic Acid Reference Standard* in alcohol to make 500 ml. Store at ca 10°. 1.0 ml = 100 micrograms of nicotinic acid.

(b) *Standard nicotinic acid stock soln II*.—To 100 ml of (a), add H_2O to make 1000 ml. Store under toluene at ca 10°. 1.0 ml = 10 micrograms of nicotinic acid.

(c) *Standard nicotinic acid soln*.—Dilute 10 ml of (b) with H_2O to make 1000 ml. 1.0 ml = 0.1 microgram of nicotinic acid. Prepare fresh standard soln for each assay.

(d) *Acid-hydrolyzed casein soln*.—Mix 100 g of vitamin-free casein with 500 ml of constant-boiling HCl soln (ca 20% HCl) and reflux the mixture for 24 hours.

¹ *This Journal*, 27, 105 (1944); 30, 82 (1947).

* U.S.P. Nicotinic Acid Reference Standard may be obtained from the U.S.P. Reference Standards, 4738 Kingsessing Ave., Philadelphia 43, Pa.

Remove the HCl from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in H_2O , adjust the soln to pH 3.5 (± 0.1) with 1 *N* NaOH soln and add H_2O to make 1000 ml. Add to the soln 20 g of activated charcoal, stir for 1 hour, and filter. Repeat the treatment with activated charcoal if the filtrate does not appear straw-colored to colorless. Store under toluene at ca 10° . Filter the soln if a precipitate forms upon storage.

(e) *Cystine-tryptophane soln.*—Suspend 4.0 g of l-cystine and 1.0 g of l-tryptophane (or 2.0 g of d, l-tryptophane) in 700–800 ml of H_2O , heat to $70-80^\circ$, and add 20 % HCl soln, dropwise, with stirring, until the solids are dissolved. Cool and add H_2O to make 1000 ml. Store under toluene at ca 10° .

(f) *Adenine-guanine-uracil soln.*—Dissolve 0.1 g each of adenine sulfate, guanine hydrochloride, and uracil in 5 ml of warm 20 % HCl soln, cool, and add H_2O to make 100 ml. Store under toluene at ca 10° .

(g) *Riboflavin-thiamine hydrochloride-biotin soln.*—Prepare a soln containing, in each ml, 20 micrograms of riboflavin, 10 micrograms of thiamine hydrochloride, and 0.04 microgram of biotin by dissolving crystalline riboflavin, crystalline thiamine hydrochloride, and crystalline biotin (free acid) in 0.02 *N* acetic acid soln. Store, protected from light, under toluene at ca 10° .

(h) *p-Aminobenzoic acid-calcium pantothenate-pyridoxine hydrochloride soln.*—Prepare a soln in neutral 25 % alcohol to contain 10 micrograms of p-aminobenzoic acid, 20 micrograms of calcium pantothenate, and 40 micrograms of pyridoxine hydrochloride in each ml. Store at ca 10° .

(i) *Salt soln A.*—Dissolve 25 g of KH_2PO_4 and 25 g of K_2HPO_4 in H_2O to make 500 ml. Add 5 drops of HCl and store under toluene.

(j) *Salt soln B.*—Dissolve 10 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.5 g of $FeSO_4 \cdot 7H_2O$, and 0.5 g of $MnSO_4 \cdot H_2O$ in H_2O to make 500 ml. Add 5 drops of HCl and store under toluene.

(k) *Basal medium stock soln.*—

Acid-hydrolyzed casein soln	= 25 ml
Cystine-tryptophane soln	= 25 ml
Dextrose, anhydrous	= 10 g
Na acetate, anhydrous	= 5.0 g
Adenine-guanine-uracil soln	= 5.0 ml
Riboflavin-thiamine-biotin soln	= 5.0 ml
p-Aminobenzoic acid-calcium pantothenate-pyridoxine soln	= 5.0 ml
Salt soln A	= 5.0 ml
Salt soln B	= 5.0 ml

Dissolve the anhydrous dextrose and Na acetate in the solns previously mixed and adjust to pH 6.8 with NaOH soln. Finally add H_2O to make 250 ml.

PREPARATION OF INOCULUM

(a) *Stock culture of Lactobacillus arabinosus 17-5.*—Dissolve 2.0 g of H_2O -soluble yeast extract in 100 ml of H_2O , add 0.5 g of anhydrous dextrose, 0.5 g of anhydrous Na acetate, and 1.5 g of agar, and heat the mixture, with stirring, on a steam bath until the agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug the tubes with cotton, sterilize in an autoclave at $121^\circ-123^\circ$ (1.1–1.2 kg per sq cm), and allow to cool in an upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of *Lactobacillus arabinosus* 17-5,² incubating for 16–24 hours at any selected temp. between 30° and 37° , but held constant to within $\pm 0.5^\circ$, and fin-

² Pure cultures of *Lactobacillus arabinosus* 17-5 may be obtained from the American Type Culture Collection, 2029 M Street, N.W., Washington 6, D. C., as Number 8014.

ally store at ca 10°. Prepare a fresh stab of the stock culture every week, and do not use for inoculum if the culture is more than 1 week old.

(b) *Culture medium*.—To each of a series of test tubes containing 5 ml of the basal medium stock soln, add 5 ml of H₂O containing 1 microgram of nicotinic acid. Plug the tubes with cotton, sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and cool.

(c) *Inoculum*.—Make a transfer of cells from the stock culture of *Lactobacillus arabinosus* 17-5 to a sterile tube containing 10 ml of culture medium. Incubate this culture for 16–24 hours at any selected temp. between 30° and 37°, but held constant to within $\pm 0.5^\circ$. The cell suspension so obtained is the inoculum.

PREPARATION OF SAMPLE SOLUTION

Place a measured amount of sample in a flask of suitable size and proceed by one of the methods given below.³

(a) *For dry or semidry materials that contain no appreciable amount of basic substances*.—Add a volume of 1.0 *N* H₂SO₄ soln equal in ml to not less than 10 times the dry weight of the sample in g, but the resulting soln shall contain not more than 5.0 mg of nicotinic acid per ml. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occurs, agitate vigorously so that all particles come in contact with the liquid, then wash down sides of flask with 1 *N* H₂SO₄ soln.

Heat the mixture in an autoclave at 121°–123° (1.1–1.2 kg per sq cm) for 30 min. and cool. If lumping occurs, agitate the mixture until particles are evenly dispersed. Adjust the mixture to pH 6.8 with NaOH soln, dilute with H₂O to make a measured volume that contains ca 0.1 microgram of nicotinic acid per ml, and filter if soln is not clear.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances*.—Add dilute H₂SO₄ and adjust the mixture to pH 6.0. Add such an amount of H₂O that the total volume of liquid shall be equal in ml to not less than 10 times the dry weight of the sample in g. Then add the equivalent of 10 ml of 10 *N* H₂SO₄ soln for each 100 ml of liquid and proceed as directed under (a).

(c) *For liquid materials*.—Adjust the material to pH 6.0 with either H₂SO₄ soln or NaOH soln and proceed as directed under (b).

DETERMINATION

Prepare standard nicotinic acid tubes as follows: To duplicate test tubes add 0.0 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml, 4.0 ml, 4.5 ml, and 5.0 ml, respectively, of the standard nicotinic acid soln. To each of these tubes add 5.0 ml of basal medium stock soln and H₂O to make 10 ml.

Prepare tubes containing the sample to be assayed as follows: To duplicate test tubes add, respectively, 1.0 ml, 2.0 ml, 3.0 ml, and 4.0 ml of the sample soln. To each of these tubes add 5.0 ml of basal medium stock soln and H₂O to make 10 ml.

After mixing, close the tubes by plugging with cotton, or covering with caps, and sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm).⁴ Cook, aseptically inoculate each tube with 1 drop of inoculum, and incubate for 72 hours at any selected temp. between 30° and 37°, but held constant to within $\pm 0.5^\circ$. Contamination of the assay tube with any foreign organism invalidates the assay.

Titrate the contents of each tube with 0.1 *N* NaOH soln, using bromothymol blue as the indicator, or to pH 6.8 measured electrometrically.

³ The concentrations of the H₂SO₄ and NaOH solns are not stated in each instance because these concentrations may be varied dependent upon the amount of sample taken for assay, volume of sample soln, and buffering effect of sample.

⁴ Overheating (oversterilizing) of the assay tubes may cause unsatisfactory results.

Prepare a standard curve of the nicotinic acid standard titrations by plotting the average of the titration values expressed in ml of 0.1 *N* NaOH soln for each level of nicotinic acid standard soln used, against micrograms of nicotinic acid contained in the respective tubes. From this standard curve, determine by interpolation the nicotinic acid content of the sample soln in each tube. Discard any values of more than 0.4 or less than 0.05 microgram of nicotinic acid in each tube. Calculate the nicotinic acid content in each ml of sample soln for each of the tubes. The nicotinic acid content of the sample is calculated from the average of the values obtained from not less than 6 of these tubes that do not vary by more than $\pm 10\%$ from the average. If the titration values of less than 6 of these tubes containing the sample soln are within the range of the titration values of the nicotinic acid standard tubes containing 0.05 to 0.4 microgram of nicotinic acid, the data are insufficient to permit calculation of nicotinic acid content of the sample. Titration values exceeding 1.0 ml for the tubes of the standard nicotinic acid soln series containing 0.0 ml of the soln indicate the presence of an excessive amount of nicotinic acid in the basal medium stock soln and invalidate the assay.

37. WATERS, BRINE, AND SALT

No additions, deletions, or other changes.

38. RADIOACTIVITY

No additions, deletions, or other changes.

39. DRUGS

(1) The following method was adopted as official, first action.

PHYSOSTIGMINE

Weigh accurately 5 g of the well mixed ointment directly into a 125 ml Erlenmeyer flask and insert a small glass rod for mixing and transfer purposes. Add 10 ml of 2% H_2SO_4 , melt the ointment completely on a steam bath and mix the contents thoroly by swirling the flask. Cool in a pan of cracked ice, swirling the contents occasionally until solidification takes place. With the aid of a glass rod, filter the acid solution thru a cotton pledget inserted in the stem of a filter funnel into a 250 ml. separatory funnel. Repeat the extraction procedure 4 more times and wash the filter funnel and cotton pledget with a few ml of distilled water. Continue as in 39.99, line 3, beginning with "Make alkaline to litmus with solid $NaHCO_3$"

(2) The tentative method for arecoline hydrobromide, 39.73 (p. 689), was adopted as official, first action.

(3) The following method was adopted as official, first action.

TRICHLOROETHYLENE

APPARATUS

Pressure Tubes

Start with a clean dry piece of soft glass tubing $16\frac{1}{2}$ cm long, 6-8 mm int. diam., with wall $1-1\frac{1}{2}$ mm thick (1 mm is easier to work). Heat in center and draw out enough to make int. diam. 3-4 mm at narrow point. Seal both ends securely by heating. When cool, cut at narrow point to make 2 tubes, which should be 8-10 mm long. Ends may instead be sealed after dividing. Narrow ends are left open.

Covered Oil Bath

Any oil bath is satisfactory which permits heating of pressure tubes in approx.

upright position and which protects analyst from burn or injury in case a tube should burst. The chief risk to guard against is that of hot oil being thrown out of bath. The following apparatus is suggested.

Wrap a large Pyrex test tube, 38×300 mm, with sheet asbestos and wire, preferably in such manner that tube may be slid in and out of wrapping. Leave round bottom exposed. Put heavy mineral oil in tube to depth of about 10 cm., or sufficient to cover closed pressure tubes. Support bath in vertical position in hood, with round bottom set into circular hole in a piece of asbestos board. A suitable thermometer will be suspended from a clamp above, with bulb immersed to vicinity of tubes, when bath is being heated.

A small cylindrical wire basket, fitting into bath tube, may be used to place tubes in bath; basket is lowered and raised by an attached wire. Basket and tubes may then be suspended in upper part of bath tube for cooling after reaction. Without basket, tubes may simply be slipped into oil bath loose, and retrieved when cooled by looped wire or other device.

In such bath several tubes may be heated at once. A smaller test tube may be used for bath if only one pressure tube is to be heated at a time.

Monoethanolamine

Colorless and free of chlorine. Commercial monoethanolamine purified by one distillation is usually satisfactory.

DETERMINATION

Tare a pressure tube with suitable support (such as a small beaker, or wire holder). Using fine-tipped pipet, place in tube 0.15–0.17 g of sample. Gently wipe away any of sample on rim or outside of tube, wait until any in upper part of tube has evaporated, and weigh. Immediately add 1.0–1.1 ml of the monoethanolamine and immediately seal open end of tube securely by means of flame, without heating liquids in bottom. When tube has cooled mix liquids completely and place in covered oil bath (see above) at room temp. Tube, or tubes, should rest in approx. upright position and remain in such position until opened later. Suspend thermometer in bath with bulb near tubes, and heat bath to 210–240°C., lowering hood window part way and observing temp. thru window. Maintain in this temp. range 1 hr. Stop heating and remove thermometer with tongs, to avoid placing hands above bath. Remove tubes from oil, but keep safely covered until cool. Use of wire basket as described above is convenient. Tubes may instead be allowed to cool in the oil (a slower procedure) and then removed. Precautions are not needed after tubes have cooled.

Remove oil from outside of each tube. Open tube by filing above liquid and breaking cleanly. With aid of H₂O wash bottle, transfer contents without loss to a 250–400 ml beaker, and dilute with H₂O to 100–120 ml. If necessary, to remove glass particles, filter thru a small cotton pledget into a second beaker, washing entire soln thru. Neutralize with HNO₃ (10–15 drops) and add 1–1.5 ml excess. Heat to 65–70°C. and add an excess of AgNO₃ soln, 5% or less (50 ml of 0.1 N, T.S., is satisfactory). Coagulate on steam bath with occasional stirring, filter thru a Gooch or fritted glass crucible, wash with hot H₂O, and dry at 130–140°C (½ hr. usually suffices) 1 g AgCl=0.30557 g. C₂H₅Cl₄.

(4) The following method was adopted as official, first action.

CALCIUM, PHOSPHORUS, AND IRON IN VITAMIN PREPARATIONS

METHOD

Transfer a representative portion of the well-mixed sample containing at least

10 mg P, 50 mg Ca, and 1 mg Fe to a 100 ml Pt or porcelain dish. Ash at a temp. not to exceed 525°C, until apparently free of carbon (gray to brown). Cool, moisten with 20 ml H₂O, break up the ash with a stirring rod, and add 10 ml HCl, cautiously under a watch-glass. Rinse off the watch-glass into the dish and evaporate to dryness on the steam bath. Add 50 ml HCl (1+9), heat on the steam bath 15 min., and filter thru quantitative paper into a 200 ml volumetric flask. Wash the filter and dish thoroly with hot water, cool the filtrate, dilute to the mark, and mix.

PHOSPHORUS

Using an aliquot containing 2–5 mg P, proceed as directed in 26.46–7.

CALCIUM

Transfer an aliquot containing 20–40 mg Ca to a suitable beaker, dilute to 100 ml., and proceed as directed in 12.12. Correct for KMnO₄ consumed in a blank determination.

IRON

Transfer an aliquot containing 0.2–0.5 mg Fe to a 100 ml volumetric flask, add sufficient HCl (1+9) to yield 2 ml of the concentrated acid, and dilute to volume. Proceed as directed in 20.12, beginning “. . . Pipet 10 ml aliquot into 25 ml volumetric flask. . . .” Determine Fe in sample by comparison with standards prepared as directed.

(5) The official method for iodine 39.202 (p. 728) was reworded as follows and adopted as official, first action:

IODINE

Transfer a quantity of sample that contains not more than 0.1 g of the iodide (0.05 g is ample) to a crucible, preferably Ni. If the sample contains only a slight amount of organic material, add one g of starch. Add 2 or 3 g of solid KOH. If sample is a solid, add 10–15 ml of alcohol before adding the KOH. (It is essential that the alkali be thoroly mixed with the sample to prevent loss of iodine in the muffle. This may be accomplished by stirring, leaving the stirring rod in the crucible, or by heating and swirling on the steam bath until the KOH is in solution.) Dry, and char thoroly. (Use as low a temp. as possible in order to prevent loss of I, in no event more than dull redness.) Extract charred mass with hot H₂O, filter into Erlenmeyer flask, and wash well with hot H₂O.

Neutralize filtrate with H₂SO₄ (1+1), make alkaline again with 4% NaOH soln, and add 1 ml in excess. Heat to boiling and add saturated KMnO₄ soln slowly until KMnO₄ color remains after several min. boiling. Then add ca 0.5 ml in excess, continue boiling ca 5 min. and allow to cool. (It is essential that the permanganate coloration, not the brown manganese dioxide color, be present at the end of the boiling period. Otherwise insufficient KMnO₄ will have been added to completely oxidize all the iodide to iodate.) Add a few ml of alcohol and place on steam bath. (KMnO₄ color should be bleached; if it is not, add a little more alcohol.) When precipitate has settled, filter and wash with hot 1% NH₄Cl soln. If the filtrate is not clear, digest on the steam bath until the MnO₂ will be retained on the filter. After cooling, add 1–2 g of KI (crystals), acidify with HCl, and titrate with 0.1 N Na₂S₂O₃ soln. 1 ml of 0.1 N Na₂S₂O₃ = 0.00277 g of KI, 0.00250 g of NaI, or 0.00212 g of I.

(6) In the last line, 39.39(b) (p. 678), the reference 39.17(b) was changed to 39.170.

40. MICROBIOLOGICAL METHODS

No additions, deletions, or other changes.

41. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

42. EXTRANEEOUS MATERIALS IN FOODS AND DRUGS

(1) The method for rodent excreta in corn meal, 42.32 (p. 781) official, first action, *This Journal*, 31, 118 (1948) was adopted as official, final action.

(2) The tentative method for filth in starch 42.38 (p. 782) was deleted and the following method was adopted as tentative.

Weigh out 225 g of starch into a 1500 ml beaker. Add with stirring 1200 ml of cold water (15–20°). Stir out lumps and pour thru a 5–8 in. #140 sieve. Wash with cold running water. Rinse particles from the sieve to a filter paper, using first water and then 80% alcohol. Examine paper microscopically.

(3) The following methods were adopted as tentative.

FILTH IN POPPED POPCORN

Procedure.—Weigh 50 g corn into a 2-liter Wildman trap flask. Add 500 ml hot water, boil for 15 min., cool to room temperature. Add 35 ml gasoline, mix. Allow to stand 5 min. Fill with H₂O, trap off, filter, and examine.

EXTERNAL CONTAMINATION OF UNPOPPED POPCORN, CEREAL GRAINS, PEAS, AND BEANS, ETC.

(1) *Macroscopic.*—Examine 225 g¹ in white tray for rodent and insect-damaged and moldy kernels, rodent excreta, other filth, and extraneous materials. Report by weight except rodent pellets. Report them by number and kind.

(2) *Microscopic.*—Transfer a separate 225² g portion to a 2-liter trap flask. Add 600 ml 40% alcohol. Boil gently, with frequent stirring, for 5 min. Cool. Trap off using gasoline and 40% alcohol, filter, and examine.

(4) The following method was adopted as tentative.

MOLD IN CRANBERRY SAUCE

(a) Strained Sauce

Immerse the unopened can of sauce in a boiling water bath for 30–45 min., in order to facilitate breaking the gel. Remove can from bath and open carefully to avoid loss of sauce thru sudden release of pressure. Empty contents of can into a suitable sized beaker (1 liter beaker for #2 can). Stir the sauce in order to break the gel. A slow-speed electric mixer (350–450 r.p.m.) may be used for this purpose.

Mix thoroly 50 g of the stirred sauce with 50 g of a 3% pectin soln. Make a mold count of this mixture using official mold count method as directed in 42.57.

(b) Whole Sauce (Seeds and Skins Included)

Pulp contents of container (if considerably greater than 1 lb, such as #10 can, remove well-mixed aliquot of 1 lb) thru cyclone with screen openings ca 0.027" in diam. This will remove skins and seeds and prepare a homogeneous pulp for mold counting. Mix 50 g of pulp with 50 g of 3% pectin soln. Make mold count of this mixture as directed in 42.57.

¹ Or consumer-size package.

² Or consumer-size package when it is 6–10 oz. Between 10–16 oz. split into two portions and test both. Over 16 oz. test an aliquot of at least 8 oz.

(5) In the method for fly eggs and maggots in tomato products, **42.61(b)** (p. 790) the "5 liter" line 4 was changed to "6 liter."

(6) In the method for filth in dried mushrooms, **42.75** (p. 795) after "mushrooms" line 10 add "allowing them to drop thru the screen."

43. STANDARD SOLUTIONS

(1) The official, first action, method for standardization of hydrochloric acid with sodium hydroxide, **43.7-43.8** (p. 803), was adopted as official, final action.

(2) The tentative method for standardization of sulfuric acid by borax, **43.14-43.15** (p. 807), was adopted as official, first action.

(3) In Method II for standardization of titanium trichloride, **21.37** (p. 290), " $K_2Cr_2O_7$ " was substituted for " $KMnO_4$ " line 1, and adopted as official, first action.

CORRECTIONS FOR VOL. 31, NO. 4

In the paper on succinic acid in decomposed egg products by Henry A. Lepper and Fred Hillig, lines 4 to 7 of paragraph 2 on page 739, beginning "The grades" and ending "Table 2" should be changed to read "The grades as indicated on the carton or on the inspector's collection report are given in Table 2 in which the other details of the samples are also summarized."

In the paper entitled "Test Paper of Urease and Acid-Base Indicator, for Detection of Urea," by J. W. Cook, on page 798, second paragraph under "Preparation of Test Papers," 4th line, after "water" insert "rendered sufficiently alkaline with dilute NaOH to dissolve the dye."

On page iii, beginning of Contents of Volume 31, change "sixty-second" to "sixty-first" annual meeting, and change the dates to "October 20, 21, and 22, 1947."*

* A corrected page is furnished in this number for purposes of binding in Vol 31. A reprint will be mailed to those who have not subscribed for Vol 32 (1949).

CONTRIBUTED PAPERS

KJELDAHL DETERMINATION OF NITROGEN IN REFRACTORY MATERIALS*

By C. O. WILLITS, M. R. COE, AND C. L. OGG (Eastern Regional Research
Laboratory† Philadelphia 18, Pennsylvania)

Since 1883, when J. Kjeldahl (9) introduced his method for the determination of nitrogen in certain compounds pertaining to the brewing industry, many improvements have been made to extend its usefulness for the analysis of nitrogen compounds in general. Investigators have studied innumerable phases of the problem with the object of increasing the accuracy and shortening the digestion time. Even today it is a subject of much interest to analytical chemists.

In the investigation reported here, a study was made of the application of the Kjeldahl procedure to heterocyclic and other aromatic nitrogen compounds generally considered refractory. Many catalysts have been proposed and used in the Kjeldahl procedure, and it was hoped that through the study of these compounds, a better evaluation of catalysts could be made. Nicotinic acid was chosen for most of these studies, since it is a representative compound of this type, can be obtained pure, and meets most of the requirements of a primary standard. Particular attention was given to the catalysts selenium, mercuric oxide, and potassium sulfate, together with times of digestion required for the determination of nitrogen by the Kjeldahl method. Neither copper nor any of its salts were included, since in previous work at this Laboratory (13), copper had no catalytic effect when used with mercury and was inferior to mercury when the two were used separately.

The use of selenium as a catalyst was first recommended by Lauro (10, 11), but later questioned by Osborn and Wilkie (14). Patel and Sreenivasan (15) studied the relationship of selenium to the recovery of nitrogen and to the loss of nitrogen. Sandstedt (18) reported low results for total nitrogen after prolonged digestion with selenium. Davis and Wise (6), Snider and Coleman (20), Dalrymple and King (5), and others reported similar losses of nitrogen, and concluded it does not appear practicable to employ selenium catalysts because digestion time would have to be determined and also controlled in order to obtain the maximum yield of nitrogen. Bradstreet (3, 4) also found progressively lower nitrogen values with increasing quantities of selenium. On the other hand, Illarionov and Soloveva (8) stated that the catalytic action of selenium was

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proportional to the amount used. Furthermore, Davis and Wise (6) observed that selenium interfered when the amount of potassium sulfate exceeded 13 g. Shirley and Becker (19) reported that a mixture of selenium oxychloride and mercury is a satisfactory catalyst for the Kjeldahl nitrogen determination of ring-type nitrogen compounds. This was likewise reported by Belcher and Godbert (2), who found that none of the catalysts they tried proved equal in efficiency to the selenium-mercury catalyst.

In addition to having an efficient catalyst for the digestion of nitrogen compounds in the Kjeldahl method, it is important to have the proper amount of potassium sulfate. The first to recognize this was Gunning (7). Later Phelps and Daudt (16) stated that the amount of potassium sulfate and sulfuric acid in the presence of mercury determines the completeness of the hydrolysis.

Alcock (1) reported experiments which showed that a definite relationship exists between sodium sulfate, sulfuric acid, and digestion time in their effect on nitrogen recovery.

PROCEDURE

REAGENTS

1. *Catalysts*.—Metallic selenium powder and mercuric oxide.
2. *Sodium hydroxide-sodium thiosulfate soln.*—Mix 100 ml of 50% sodium hydroxide and 25 ml of 8% sodium thiosulfate soln.
3. *Indicator*.—Mix 400 ml of 0.1% methyl red dissolved in 95% ethanol with 100 ml of 0.1% methylene blue also dissolved in 95% ethanol.
4. *Boric acid*.—Dissolve 4 g of boric acid in 100 ml of distilled water.
5. *Standard acid*.—0.1 *N* hydrochloric acid.

The Kjeldahl method followed differed little from the one in common use. In addition to the sample, the digestion mixture consisted of sulfuric acid, potassium sulfate and 2 catalysts. A sample containing at least 30 mg. of nitrogen was used so that 20 ml. or more of standard 0.1 *N* acid would be required in the titration. In all cases, the digestion was made in a 650-ml. Kjeldahl flask with 25 ml. of sulfuric acid, to which was added different amounts of potassium sulfate, mercuric oxide, and selenium. The mixture was digested for 1 to 8 hours with full boiling; chips were used to maintain uniform ebullition. The time of boiling had no relation to the time required to clear the solution.

The digest was diluted with 250 ml. of distilled water, and made alkaline with sodium hydroxide-sodium thiosulfate solution; 5 g. of 20-mesh zinc granules were then added. The distilling apparatus used a modified connecting bulb (21) and a 500-ml. wide-mouth Erlenmeyer receiving flask sealed with a trap (17). The condenser tube extended below the surface of the 100 ml. of 4% boric acid solution used as the trapping liquid.

RESULTS

Figure 1 shows the effect on the recovery of nitrogen in nicotinic acid of varying amounts of selenium with three different weights of potassium sulfate, with and without 0.4 g. of mercuric oxide. A digestion time of 6 hours was used.

These results indicate that to obtain theoretical recoveries of nitrogen with 5.5 g. of potassium sulfate, the optimum amount of selenium was between 0.25 g. and 0.40 g., whereas with 11.0 or 16.5 g. of potassium sulfate, less than 0.015 g. of selenium could be used. In the absence of

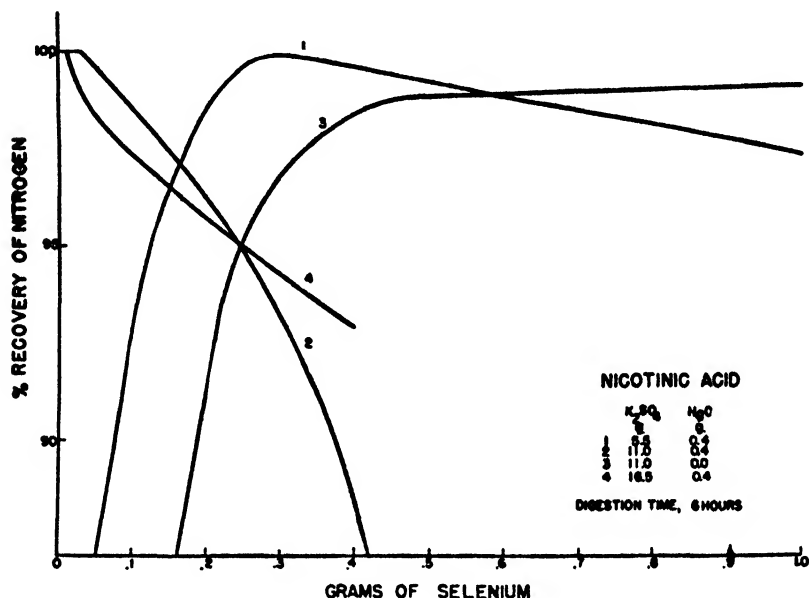


FIG. 1.—Recovery of nitrogen as affected by varying amounts of selenium with different amounts of potassium sulfate.

mercury, as much as 1.50 g. of selenium to 11 g. of potassium sulfate gave incomplete recovery.

These results indicated that some optimum ratio of selenium to potassium sulfate might be found. To determine this, studies were made with different amounts of potassium sulfate and 0.3, 0.03 and 0.015 g. of selenium. Figure 2 shows that the optimum amount of selenium decreases as the amount of potassium sulfate increases. However, with a prolonged digestion time, in this case 6 hours, it was easily possible to exceed the optimum amount of potassium sulfate to be used with 0.3 g. or 0.03 g. of selenium, with a resultant loss of nitrogen. The long digestion period of 6 hours was chosen so that any loss of nitrogen would be recognized as such and not confused with incomplete digestion. The use of 0.015 g.

of selenium had little influence, since the recovery of nitrogen was essentially the same as when no selenium was used. Three-hundredths gram of selenium gave maximum recoveries when used with 11 g. of potassium sulfate but caused a loss of nitrogen with larger amounts. This increasing loss of nitrogen with increasing amounts of potassium sulfate, as would be expected, was more pronounced when larger amounts (0.3 g.) of selenium were used.

From the preceding experiments, it appeared desirable to establish if, when large amounts of selenium (0.30 g.) and varying amounts of potas-

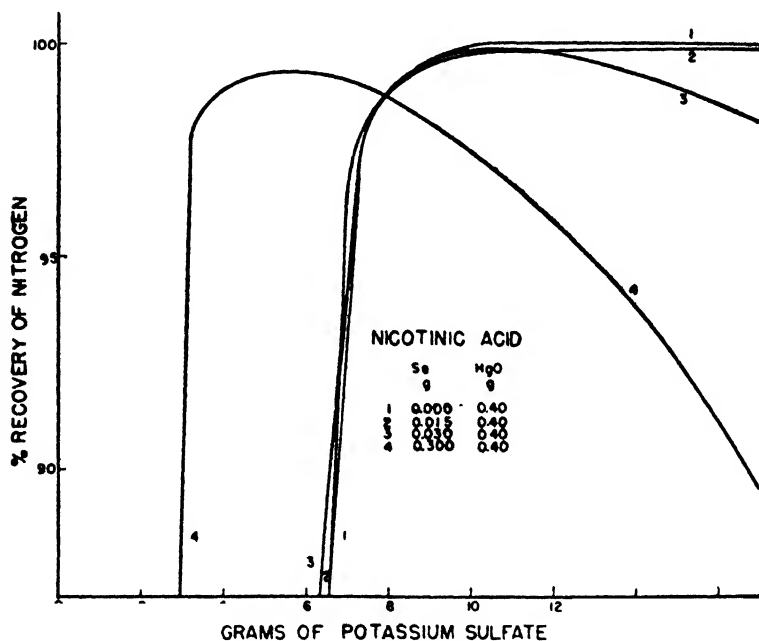


FIG. 2.—Recovery of nitrogen as affected by varying amounts of potassium sulfate with different amounts of selenium.

sium sulfate are used, there is some specific digestion time in which theoretical or maximum recoveries of nitrogen can be obtained. Figure 3 shows that for mixtures containing 0.3 g. of selenium and 5.5 g. of potassium sulfate, 99.8% of the nitrogen of nicotinic acid is recovered after 6 hours digestion, and that there is no apparent loss of nitrogen after 7 and 8 hours. With larger amounts of potassium sulfate, 11 to 15 g., the maximum but less than theoretical recovery of nitrogen occurs with a digestion time of between 2 and 4 hours, and a loss of nitrogen occurs with longer periods. Since the maximum recovery with 11 or more g. of potassium sulfate and 0.30 g. of selenium was less than theoretical, and since the recovery diminished with longer digestion periods, it was decided that

for all future experiments a smaller amount (0.03 g.) of selenium should be used.

It was indicated in Figure 2 that theoretical recoveries of nitrogen could be had without the use of selenium, but there was no indication as to the effect of digestion time on the recovery. A series of determinations was therefore made with varying amounts of potassium sulfate, and 0.4 g. of mercuric oxide with digestion times of 1 to 8 hours. A parallel experiment was made in which 0.03 g. of selenium was added to the digestion

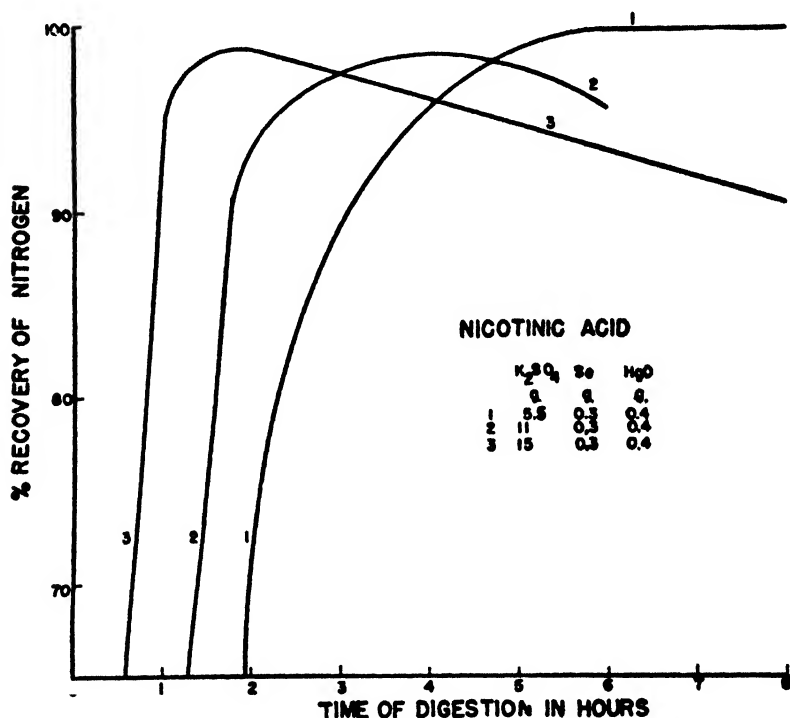


FIG. 3.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of selenium.

mixture. The results of these two experiments are shown in Figures 4 and 5, respectively.

A comparison of Figures 4 and 5 shows that the best digestion mixture contained 15 g. of potassium sulfate, 0.4 g. of mercuric oxide, and no selenium, since it required the shortest digestion period (2 hours) for complete recovery of nitrogen and prolonged digestion caused no loss of nitrogen. With low concentration of sulfate salts, selenium caused a somewhat more rapid rate of nitrogen recovery, whereas with higher concentrations the reverse was true.

In all the preceding experiments, the amount of mercuric oxide, 0.4

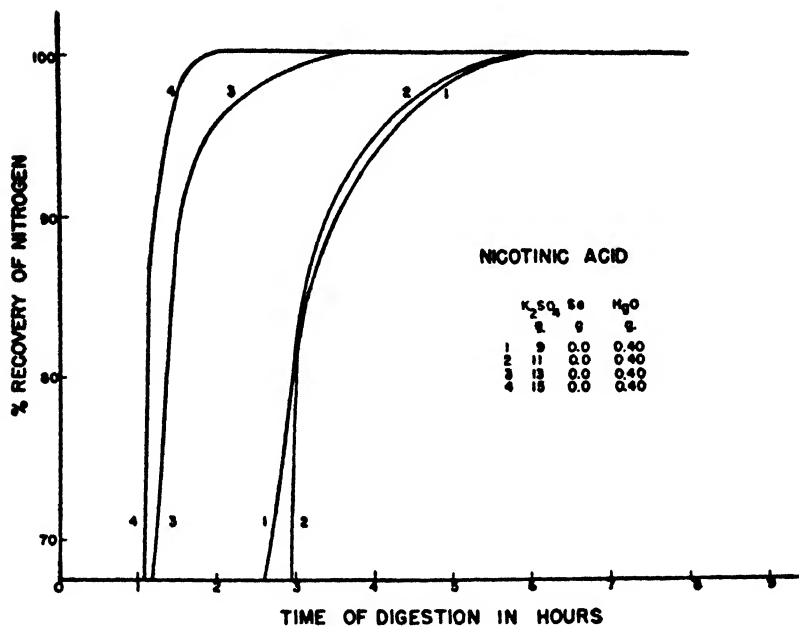


FIG. 4.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of potassium sulfate, without selenium.

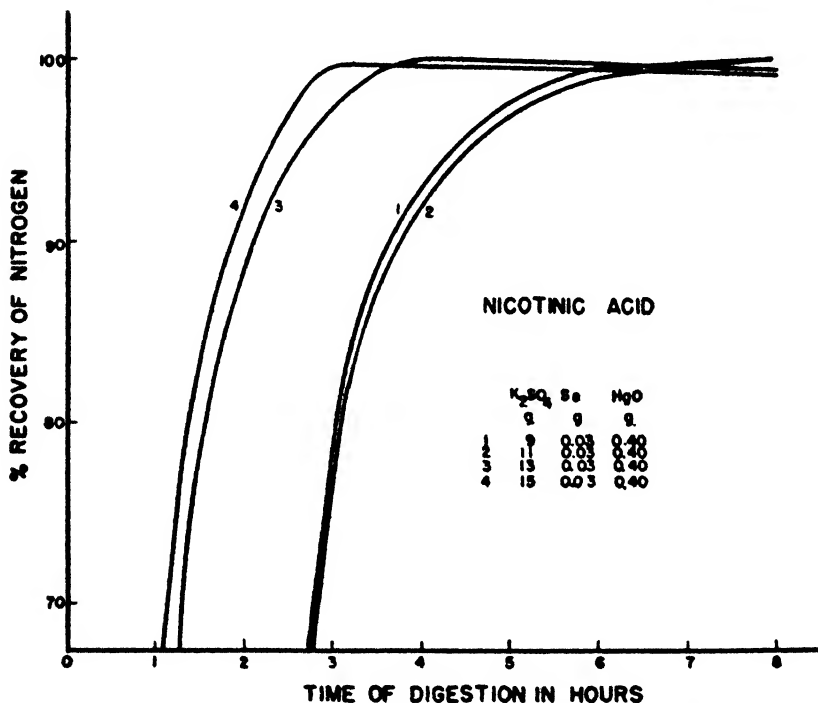


FIG. 5.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of potassium sulfate with selenium in the mixture.

g., was chosen arbitrarily. Experiments were therefore made, as shown in Figure 6, to determine the effect of varying the amount of mercuric oxide. For digestion mixtures containing 15 g. of potassium sulfate and *no mercuric oxide*, less than 55% of the nitrogen was recovered with a 3-hour digestion. The amount of mercuric oxide required with this quantity of potassium sulfate to give complete recovery of nitrogen was not critical, since as little as 0.1 g. or as much as 1 g. gave equally satisfactory results.

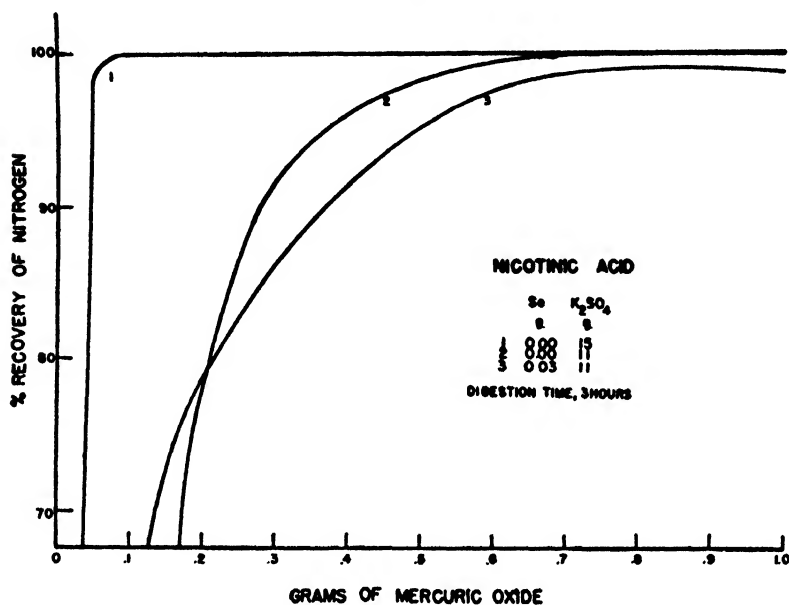


FIG. 6.—Recovery of nitrogen as affected by varying the amounts of mercuric oxide with different amounts of selenium and potassium sulfate.

For the smaller amount of potassium sulfate, theoretical recoveries were obtained only when more than 0.6 g. of mercuric oxide was used. The presence of selenium prevented the complete recovery of nitrogen with all amounts of mercuric oxide used.

Table 1 gives the results of the analyses of other refractory nitrogen compounds. With the digestion mixture consisting of 15 g. of potassium sulfate, 0.4 g. of mercuric oxide, and 25 ml. of sulfuric acid, and a digestion time of 3 hours, the amounts of nitrogen recovered were in close agreement with the theoretical values.

The preceding experiments showed that a simple digestion mixture of potassium sulfate, mercuric oxide, and sulfuric acid was sufficient for refractory compounds, but it was not certain what would happen to a less refractory material when digested for 3 hours or longer. Table 2 shows the recovery of nitrogen from S-benzyl thiuronium chloride with $\frac{1}{2}$ - to 6-hour digestion periods. Complete recovery of nitrogen was ob-

TABLE 1.—*Per cent nitrogen found in several typical refractory nitrogenous compounds*

	NITROGEN		
	FOUND BY ANALYSIS		THEORETICAL VALUE
	A	B	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Tryptophane	13.60	13.60	13.72
Histidine monohydrochloride	19.94	19.93	20.05
Lysine monohydrochloride	15.24	15.24	15.34
8-hydroxy quinoline	9.61	9.62	9.65
Nicotinic acid	11.38	11.40	11.38

TABLE 2.—*Per cent nitrogen recovered from S-benzyl thiuronium chloride* using the potassium sulfate-mercuric oxide digestion mixture*

DIGESTION TIME, HOURS	NITROGEN RECOVERED
	<i>per cent</i>
0.5	13.81
1	13.82
2	13.85
3	13.83
4	13.84
5	13.82
6	13.83

* Theoretical nitrogen value, 13.82.

tained after half an hour of digestion, and there was no appreciable loss up to 6 hours.

DISCUSSION

These experiments have demonstrated that the time of digestion and the amounts of potassium sulfate and of mercuric oxide used have an interrelated effect on the loss of nitrogen when selenium is present. This may be the reason that many have inaccurately defined the cause of incomplete recovery of nitrogen when selenium was used in the digestion mixture. Since selenium (1) may cause either incomplete recovery of nitrogen or a diminished rate of recovery, and (2) requires a specific time of digestion, its use cannot be recommended.

When selenium is omitted from the digestion mixture, the amount of potassium sulfate, mercuric oxide, and the time of digestion are not critical, insofar as loss of nitrogen is concerned. It was possible, therefore, to increase the amount of potassium sulfate so that complete recovery was obtained in 2 hours with no loss of nitrogen on prolonged digestion.

For the complete recovery of nitrogen from all kinds of materials which may contain refractory nitrogenous compounds, it is recommended as a general procedure that a digestion mixture consisting of 25 ml. of sulfuric acid, 15 g. of potassium sulfate, and 0.6 g. of mercuric oxide be used, with a digestion time of 3 hours. This method, which is a result of the studies reported here, is essentially the same as the A.O.A.C. method (12), and is further proof of its general applicability. If the 15 g. of potassium sulfate is carefully measured, 0.4 g. of mercuric oxide is more than sufficient, as shown in Figure 6. However, if less than 15 g. is added, as is often the case, 0.6 g. of mercuric oxide should be used.

In Figures 4 and 5 the curves for 9 and 11 g. of potassium sulfate, both with and without selenium, almost coincide, whereas the similar paired curves for 13 and 15 g. are markedly different. This peculiarity may explain why different analysts, using presumably the same Kjeldahl method, often obtain different results for refractory compounds. Since potassium sulfate is usually measured by volume instead of by weight, it would be possible for one analyst to add 13 and the other 11 g., and each think that he was following the procedure rigorously. Should both use a 4-hour digestion, one would obtain complete recovery and the other only 92 to 95% recovery.

The desirability of using a mercury compound as a catalyst is beyond question. The omission of mercuric oxide from the digestion mixture always causes incomplete recovery of nitrogen from nicotinic acid. With high concentrations of potassium sulfate, as little as 0.1 g. of mercuric oxide is sufficient.

SUMMARY

Complete recovery of nitrogen can be obtained from heterocyclic nitrogen ring compounds by the Kjeldahl method. The only catalysts required are mercuric oxide and potassium sulfate, used in the ratio of 0.6 to 15 g., with 25 ml. of sulfuric acid, and a digestion time of 3 hours. Because of the danger of loss of nitrogen when selenium is present, the use of selenium as an additional catalyst cannot be recommended.

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A NEW METHOD FOR DETERMINING THE BOILING RANGE OF PSEUDOCUMIDINE IN FD&C RED NO. 1*

By KENNETH A. FREEMAN AND LEE S. HARROW (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

FD&C Red No. 1 is one of the colors certifiable by the U. S. Food and Drug Administration for use in foods, drugs, and cosmetics. In order to be certified, the color must meet the specifications set forth in the Coal-Tar Color Regulations (1). One of the specifications states that the boiling range of crude pseudocumidine used in the production of the color, or of the pseudocumidine obtained by the reduction of the color, must be in the range of 220-245°C.

The Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition (2) gives a tentative method for this determination. This method involves reduction of the FD&C Red No. 1 with stannous chloride and hydrochloric acid, addition of excess sodium hydroxide, and separation of the free amine by means of steam distillation. The amine is finally recovered by ether extraction from the distillate. Experience has shown several objectionable features in this method.

- (a) Irritating HCl fumes are produced during the reduction.
- (b) Unless the addition of sodium hydroxide to the reduction mixture is very cautiously carried out the reaction mixture spatters.
- (c) Low yields of pseudocumidine are produced.
- (d) The method is very time-consuming.

In order to overcome these objections, a means was sought for making this determination by another method. Mr. L. Koch (3) of H. Kohnstamm and Company, Inc., suggested the use of sodium hydrosulfite in alkaline solution as the reducing agent. This procedure was found to be superior to the stannous chloride method. Certain modifications were made to provide simultaneous reduction of the color and recovery of the pseudo-

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11-13, 1948.

cumidine. A modified semi-micro distillation procedure for determining the boiling range of the pseudocumidine has also been developed.

METHOD APPARATUS

Steam distillation.—A three-liter, two-neck, round bottom flask; one neck is fitted with a dropping funnel; the other with a steam distillation trap which has an inlet for live steam and which is connected to a water-cooled condenser.

Fractionating apparatus.—A 25 ml round-bottom flask fitted with an insulated distilling column, 10–12" long and $\frac{1}{4}$ " inside diam., packed with 24" of approximately 24 gauge nichrome wire coiled inside the column in the manner described by Podbielniak (4). An insulated Vigreux column of approximately the same dimensions is also suitable. Provide the column with a 200–260°C Anschütz thermometer.

REAGENTS

Sodium hydrosulfite.—Reagent grade, saturated soln (ca 30%).

Dissolve ca 100 g of color in 2 liters of hot water contained in the round-bottom flask of the steam distillation apparatus. Add 10 g of sodium hydroxide and heat the soln to boiling, then while passing live steam thru the soln at a rate that will produce 5–10 ml of distillate per min., add the sodium hydrosulfite soln dropwise by means of the dropping funnel until the red color disappears (the soln will then be a light yellow-brown). Continue the steam distillation until no more oil distills. Extract the distillate with four 20 ml portions of ether and wash the extracts several times with 10 ml portions of water. Evaporate the major portion of the ether on a steam bath and dry the residue over sodium or potassium hydroxide pellets. Filter the residual soln into the 25 ml round-bottom flask of the fractionating apparatus. Heat cautiously with a heating mantle or a water bath until all the ether has been removed; then continue heating with a Wood's metal bath or an equivalent constant temperature bath. Record the first point of constancy in the temperature rise as the initial boiling point. The final point is taken as the maximum temp. obtainable.

EXPERIMENTAL

A commercial sample of "liquid pseudocumidine" was distilled and the fraction boiling from approximately 218–240°C. was reserved. This material was kept for 24 hours over sodium hydroxide pellets and then divided into two portions. The boiling range of one portion was determined using the apparatus suggested under "Method."

A quantity of FD&C Red No. 1 was prepared from the second portion employing the following procedure:

Dissolve 27 g of crude pseudocumidine (0.2 mol) in 150 ml of 6 *N* HCl contained in a 600 ml beaker. Place the beaker in an ice bath and add 65 g of crushed ice to the soln. Add 100 ml of a cold 21% soln of sodium nitrite, and hold at 5°C. or below. At the end of one hour add small portions of sulfamic acid until a negative test is obtained with starch-iodide paper.

Place in a 2-liter beaker 15 g of sodium carbonate, 15 g of sodium acetate, 6 ml of 30% sodium hydroxide, 67 g of R-salt, and 1 liter of water. When all material is in solution, cool to 5°C. and add the cold diazonium soln from above. Stir for 30 min. holding the temp. at 5°C. or below; then allow the soln to warm to room temp. Heat on steam bath for four hours, cool, and filter. Dry the dye at 135°C. for six hours. By this method a yield of 86 g was obtained (86% of theory).

This color was then reduced according to the proposed method and the boiling range of the resulting pseudocumidine was determined. The results are shown in Table 1.

TABLE 1

BOILING RANGE	% DISTILLED ORIGINAL	RECOVERED MATERIAL
Initial-220°C.	8	10
220-225°C.	55	50
225-227°C.	17	20
227-Upper Limit	19	20

The boiling range of the original material was 218.0°C. to 232.4°C. and that of the recovered material was 218.2°C. to 231.9°C.

DISCUSSION

The proposed method has been employed in the Color Certification laboratories for a period of several months. This experience has shown that the method eliminates the objections of the older method. All of the non-volatile reduction products are soluble in the solution employed. The use of hydrochloric acid is avoided entirely. Neutralization of the reduction mixture is not necessary because the reduction is accomplished in basic solution. The time consumed is greatly reduced, since the reduction of the dye and distillation of the pseudocumidine are occurring simultaneously.

The entire reduction and steam distillation process is carried out in a single piece of apparatus, thus eliminating transfer of material from one apparatus to another (with the consequent loss of material and time consumption).

SUMMARY

A method has been proposed for the evaluation of the boiling range of pseudocumidine in FD&C Red No. 1 (Ponceau 3R). The intermediate is recovered from the color by reduction with alkaline sodium hydrosulfite. The boiling range is determined by a semi-micro fractionating apparatus fitted with an Anschütz thermometer. Experience has shown that the method is more reliable, shorter, and more convenient than the method given in *Methods of Analysis*.

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SPECTROPHOTOMETRIC ANALYSIS OF D&C RED No. 19 (RHODAMINE B)*

By MEYER DOLINSKY (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

One of the specifications (1) for certifiable samples of D&C Red No. 19 is: "Pure dye (as determined by titration with titanium trichloride), not less than 92.0 per cent." The titanium trichloride titration is not very satisfactory since it gives variable, and generally high, results. It was decided, therefore, to study the spectrophotometric analysis of this color, since it appeared that the spectrophotometric determination might be more accurate and precise than the titration method.

As in previous investigations (2) (3), samples sufficiently pure to serve as standards were prepared and spectrophotometric data obtained on solutions of these samples. From these data, the applicability of Beer's law was checked, the location of the absorption peak was determined, and the extinction ratio at arbitrarily selected wave lengths was calculated as an aid in the identification of the color.

EXPERIMENTAL

All optical measurements were made with a General Electric recording spectrophotometer equipped with slit adjustments for an 8 millimicron wave length band. To minimize the effect of the fluorescence of the dye, the cells containing the solutions used in this work were placed at the forward end of the transmission compartment, approximately five inches from the integrating sphere. Calculations indicate that under these conditions less than one per cent of the fluorescent light emitted by the sample should reach the integrating sphere.

Melting points were taken on a Fisher melting point block.

Preparation of Standard Samples.—30 grams of phthalic anhydride (sublimed), m.p. 130.5°C. (literature, 130.8°C.), was fused with 24 grams of m-diethylaminophenol (recrystallized twice from methanol-water and once from chloroform-petroleum ether), m.p. 72–72.5°C. (literature 76°C.), at 165°C. for five hours (4). The Rhodamine B phthalate obtained was treated with ammonium hydroxide and the color base extracted from the ammonium hydroxide solution with several portions of benzene. The D&C Red No. 19 was extracted from the benzene with several portions of hot (1+4) hydrochloric acid and allowed to crystallize.

(a) Part of the D&C Red No. 19 was recrystallized twice from (1+8) HCl; once from (1+20) HCl, and dried at 80°C. to give a product which softened at 185°C. and melted at 193–195°C.

(b) A portion of the crude D&C Red No. 19 was converted to the color

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base by precipitation from solution with sodium hydroxide. The base was recrystallized three times from ether and dried at 135°C. to give a practically colorless (very light pink) product which melted sharply at 165°C. (literature 165°C.). (5) A separate portion of the color base recrystallized twice from methanol-water had the same melting point and showed identical spectrophotometric characteristics.

(c) Part of the crude Rhodamine B phthalate was recrystallized three times from alcohol and dried at 135°C. to give a product melting at 212°-214°C.

Analytical data on the purified compounds are shown in Table 1.

TABLE 1.—*Analytical data*

	NITROGEN (KJELDAHL)		CHLORINE	
	CALCULATED	FOUND	CALCULATED	FOUND
	per cent	per cent	per cent	per cent
Purified D&C Red No. 19	5.85	5.79	7.4	7.3
Purified D&C Red No. 19 Color Base	6.33	6.30		
Purified Rhodamine B Phthalate	4.60	4.55		

Spectrophotometric Data

(a) A sample of the purified color base, weighed on a semimicro balance, was dissolved in exactly 1000 ml. of (1+999) HCl. Aliquot portions of this solution were diluted with (1+99) NH₄OH to give the solutions for spectrophotometric examination. (All solutions were allowed to stand for approximately one hour at the temperature of the room in which the optical measurements were made, before being made to volume.) Typical results are shown in Table 2 and typical curves in Figure 1.

TABLE 2.—*Extinction values of purified D&C Red No. 19 color base in dilute ammonia*

CURVE NO. (FIG. 1)	CONCENTRATION MG. LITER	E _{553mμ}	E _{553mμ}
			CONCENTRATION
1	1.03	0.260	0.252
2	2.06	0.516	0.251
3	4.11	1.033	0.251
		Average	0.251
Calculated as the hydrochloride		Average	0.233

(Extinction values are corrected for a Signal Lunar White Glass H-6946236 reading of 1.050.)

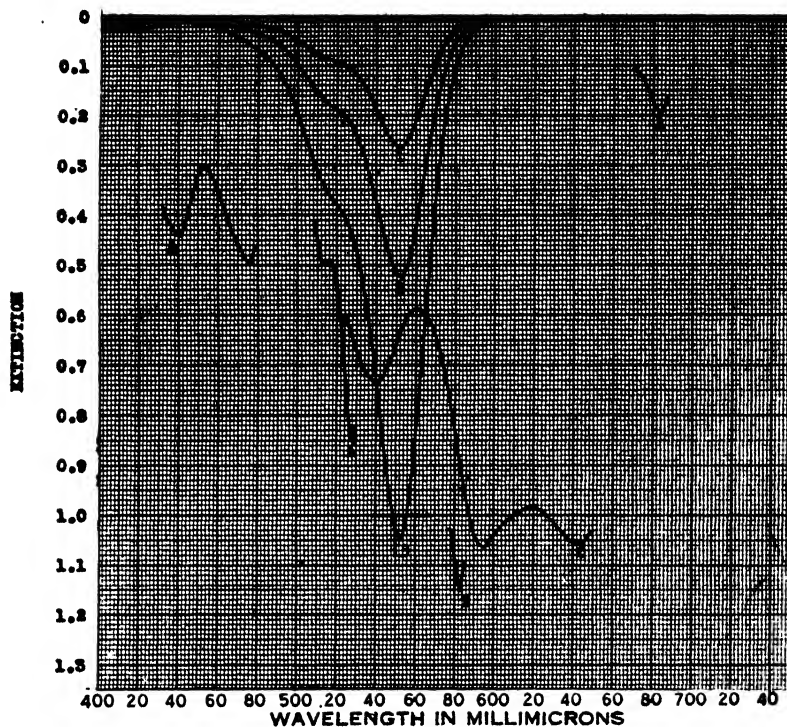


FIG. 1.—Absorption Curves of Purified D&C Red No. 19 Color Base in Dilute Ammonia.

Curve 1—1.03 mg./liter.

Curve 3—4.11 mg./liter.

Curve 2—2.06 mg./liter.

Cells —1 cm.

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$).

B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 $m\mu$).

C = Signal Lunar White Glass-H-6946236.

(b) A sample of the purified D&C Red No. 19, weighed on a semimicro balance, was dissolved in exactly 1000 ml. of distilled water. Aliquot portions of this solution were then diluted with (1+99) NH_4OH to give the solutions for spectrophotometric examination. Typical results are shown in Table 3.

TABLE 3.—Extinction values of purified D&C Red No. 19 in dilute ammonia

CURVE NO.	CONCENTRATION MG./LITER	$E_{583m\mu}$	$E_{583m\mu}$
			CONCENTRATION
1	1.18	0.278	0.236
2	2.37	0.553	0.233
3	4.73	1.093	0.231
		Average.....	0.233

(c) A sample of the purified Rhodamine B phthalate weighed on a semimicro balance, was dissolved in exactly 1000 ml. of (1+99) HCl. Aliquot portions of this solution were then diluted with (1+49) NH_4OH to give the solutions for spectrophotometric examination. Typical results are shown in Table 4.

TABLE 4.—*Extinction values of purified Rhodamine B phthalate in dilute ammonia*

CURVE NO.	CONCENTRATION MG./LITER	$E_{553m\mu}$	$E_{553m\mu}$
			CONCENTRATION
1	1.54	0.282	0.183
2	3.08	0.564	0.183
3	6.15	1.118	0.182
		Average	0.183
Calculated as hydrochloride		Average	0.232

(d) To equal aliquots of a master solution of the color, acid, alkali or buffer was added and the solution then diluted to a definite volume. The pH values of these solutions were determined, using a glass electrode pH meter, and the absorption curve run. The results are given in Table 5 and Figure 2.

TABLE 5.—*Absorption curves of D&C Red No. 19 in aqueous solution at various pH levels (conc. = 3.66 mg./liter)*

CURVE NO. (FIG. 2)	pH	ABSORPTION PEAK	$E_{\text{Absorption Maximum}}$
			CONCENTRATION
1	13.1	553	0.233
2	7.1	553	0.233
3	6.0	553	0.233
4	3.5	554	0.228
5	1.4	556	0.206

DISCUSSION

The absorption curve of D&C Red No. 19 in neutral or basic solution shows a sharp peak at $553 \pm 2 m\mu$ with a "shoulder" at $520 m\mu$. In strongly acid solution the peak shifts to $556 m\mu$ and the extinction per milligram is slightly lower.

Solutions of the color in dilute ammonia follow Beer's law to within $\pm 0.36\%$ if the effect of the fluorescence of the solutions is eliminated. When the effect of the fluorescence is not eliminated, the results at concentrations giving a density of about 1.0 may deviate as much as 4% from Beer's law. The average extinction per milligram per liter for the color in dilute ammonia solution at $553 m\mu$ was found to be 0.233. This figure is

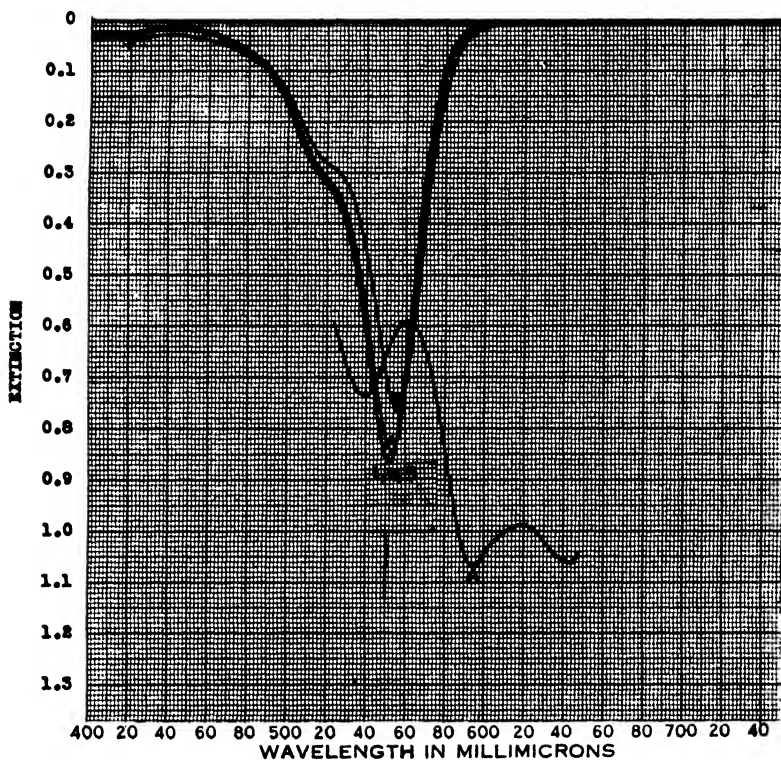


FIG. 2.—Absorption Curves of D&C Red No. 19 in Aqueous Solution at Various pH Levels (Conc. = 3.66 mg./liter).

Curve 1—pH 13.1

Curve 2—pH 7.1

Curve 3—pH 6.0

Curve 4—pH 3.5

Curve 5—pH 1.4

Cells —1 cm.

A = Signal Lunar White Glass-H-6946236.

based on the results of twenty-five determinations. The average deviation from this figure was 0.43% and the maximum deviation 1.07%.

Variation of the amount of concentrated ammonium hydroxide in the solution for spectrophotometric analysis from 1 ml to 10 ml per 100 ml of solution did not change the spectrophotometric characteristics. Solutions stored for twenty-four hours gave curves identical with those of freshly prepared solutions.

APPLICATION TO COMMERCIAL SAMPLES

Three commercial samples of D&C Red No. 19 were analyzed spectrophotometrically, following the procedure described for the standard samples. The data are shown in Table 6.

A commercial sample of D&C Red No. 19, Aluminum Lake, was analyzed spectrophotometrically by dissolving a sample in (1+19) HCl and

TABLE 6.—*Analysis of commercial samples of D&C Red No. 19*

SAMPLE	CONCENTRATION MG./LITER	$E_{553m\mu}$ (IN DILUTE AMMONIA)	DYE SPECTROPHOTO- METRICALLY	DYE BY TITRATION WITH $TiCl_3$	DYE FROM NITROGEN CONTENT
D&C Red No. 19	4.0	0.901	per cent 96.6	per cent Variable (97–105)	per cent 96.8
D&C Red No. 19	4.0	0.886	95.0	98.5	94.4
D&C Red No. 19	4.0	0.904	97.0	95.6	99.6
D&C Red No. 19 Al. Lake	20.0	1.126	24.2	23.0	—

diluting the solution with (1+99) NH_4OH to a suitable concentration of the dye. The result obtained is included in Table 6.

SUMMARY

Spectrophotometric data obtained from dilute aqueous solutions of purified D&C Red No. 19 are presented. Beer's law is shown to be applicable to dilute ammoniacal solutions containing one to five mg. per liter of dye. The average extinction per mg. per liter is 0.233 ± 0.001 at $553 m\mu$. The extinction ratio $E_{520m\mu}/E_{553m\mu}$ is 0.36 ± 0.01 . These data are applied to the determination of "pure color" in commercial samples of the color and a color lake.

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DETERMINATION OF CARBON BLACK IN CHOCOLATE

By ALBERT P. SIDARAS (Walter Baker Chocolate and Cocoa Division of General Foods, Dorchester 24, Massachusetts)

SUMMARY

A method has long been needed for the determination and detection of carbon black in chocolate (when present). Such a method has been developed at the Walter Baker Laboratory.

This procedure for carbon black detection and quantitative estimation

has proven to be very satisfactory. It involves nitration, acetylation, and solvent washing, which leaves the carbon free so that it can be determined by gravimetric analysis. This method is sensitive to one part carbon black in 100,000 parts of chocolate.

INTRODUCTION

For some time some chocolate manufacturers had used carbon black in the formulation of their products, the purpose of which was to darken the chocolate without the use of dutched liquor, which imparted an undesirable flavor. The standard for chocolate products adopted by the Federal Security Administration on Dec. 6, 1944, do not include carbon black as an ingredient. For this reason it becomes an adulterant and a method for its detection and determination is required.

It was originally thought that if the cacao coloring matter could be easily removed from the chocolate, then the carbon black in the chocolate could be detected microscopically. With this in mind the following two approaches to the problem were tried but proved to be unsuccessful.

(A) Digestion with acid and alkali similar to the crude fiber procedure but with increased concentration of acid and alkali and longer digestion periods.

(B) Successive digestion with various solvents, viz, (1) diethyl ether, (2) water Soxhlet extraction, (3) acidified alcohol Soxhlet extraction, (4) refluxing with 5% sodium hydroxide, centrifuging, and decanting liquor; repeating the sodium hydroxide operation with the residue until the residue becomes colorless.

At this point it was realized that an entirely different approach would be necessary and to detect carbon all other matter would have to be removed. To this end the following three techniques were tried. These techniques were based on procedures used to determine carbon black in rubber.*

(A) Nitration with nitric acid (after fat removal) followed by water flotation.

(B) Nitration with nitric acid followed by potassium hydroxide fusion.

(C) Nitration with nitric acid followed by acetylation.

Procedure (A) was satisfactory for a qualitative but not for a quantitative test and, therefore, was discarded.

Procedure (B) was too hazardous and costly so was not given further consideration.

Procedure (C) was the method that was finally adopted as most suitable.

METHOD

Introduce into a 250 ml. flask 100 gm. of the chocolate sample in a molten state,

* *Ind. Eng. Chem. Anal. Ed.*, 9, 278 (1937).

and of temp. ca 40°C. To this add 100 ml of petroleum ether, stopper, and shake the flask until chocolate is completely dispersed in ether. Filter this mixture thru an S&S #589 Black Ribbon paper (15 cm) in a Buchner funnel. (This filter paper should be moistened first with distilled water, the excess of which should be drawn off, as it tends to retard the filtration rate.) Turn off the vacuum after the first portion of ether has passed thru. Soak the residue in the funnel and wash with 3 successive 100 ml portions of petroleum ether, and draw off the ether by vacuum after each soaking and washing. Dry the filter cake by leaving the vacuum on after the final washing. (At this point, the residue should be completely defatted. If there should be a small amount of petroleum ether present in the filter cake it will not interfere with the nitration procedure to follow.) Transfer the filter cake and paper (the paper will not interfere with the nitration) from the funnel quantitatively into a 2-liter beaker and break the filter cake into lumps the size of a pea (Hood Operation). Add 100 ml of 16 normal nitric acid all at once. Stir this mass until it is completely mixed with the acid. In about 4-5 min., the mass will start to froth and give off nitrogen dioxide. At this point place the beaker into an ice bath until the frothing stops. During this period, occasional stirring will be required so as to prevent the froth from climbing over the sides of the beaker. When all activity has ceased, remove the beaker from the ice bath (9-13 min.) and place it in a hot water bath (100°C.). Cover the beaker with a watch-glass and allow the digestion to continue until very little nitrogen dioxide fumes are given off. When this point is reached, fill a 100 ml pipet with 16 normal nitric acid and wash down the side of the beaker and the stirring rod. When the acid wash is completed, cover the beaker again and continue the acid digestion on the water bath until a small volume of fumes is given off. When this occurs, remove the beaker from the hot water bath and dilute the nitration mixture with 100 ml. of cold, distilled water. Filter this mixture completely thru an S&S #589 Green Ribbon filter paper (18.5 cm) in a Bunsen funnel. When the filtration is complete, wash the residue 3 times only, with boiling distilled water. (A fourth washing generally starts to plug the paper.) During these washings, direct the jet of water at the bottom of the filter so as to break up the pulpy skin that forms on the lower part of the filter paper. (Do not try using vacuum for this filtration as it will plug the filter.)

(If the residue at this point is gray or black, then there is definite indication that carbon black is present. For qualitative work, the procedure carried this far would be sufficient to indicate carbon.)

For quantitative results, scrape the residue from the filter paper in the qualitative procedure into a 300 ml glass-stoppered iodine number flask (Cenco #14920), and wash the remaining material on the paper into the flask with a jet of diethyl ether. Evaporate the ether on a hot water bath, using a dry air jet to facilitate the removal of ether fumes. When the evaporation is complete, scrape down the sides of the flask and finish drying any retained moisture in an oven at 120°C.

When the residue is thoroly dry, add a cold mixture of the following reagents which have been previously mixed: 75 ml 95.5% acetic acid; 75 ml acetic anhydride; and 10 ml of sulphuric acid (conc.). Stopper the flask and with occasional shaking let this mixture stand at room temp. or at an elevated temperature of 43°C. until the pulp has made complete solution. At this point, the analyst should be absolutely certain that this pulp is in complete solution for, unless it is, it will make an error in the amount of carbon black finally determined. (Several hours may be required for complete acetylation.)

Dilute this acetylated mixture with 75 ml of 99.5% of acetic acid and filter thru a Gooch crucible which has a heavy asbestos mat. (This crucible should have been previously ignited at 800°C.) After this mixture has completely filtered, wash it with 99.5% acetic acid until the filtrate shows no color, then wash it twice with

chloroform and only twice with boiling, distilled water. Dry at 120°C. for two hours or until constant. Cool in desiccator and weigh.

Ignite the crucible and residue from the acetylation in a muffle furnace at 800°C. for two hours after the furnace has come up to temperature. At the end of the ignition period, remove the crucible to a desiccator and allow it to cool, and weigh for loss. Subtract this weight from the previous weight, and the loss in weight is the carbon if all the pulp has gone into solution previously.

DISCUSSION

To save time and avoid possible error it was first thought that the filter paper might be included in the acetylation procedure. But when this procedure was followed it was found to be objectionable because of the increase in digestion time and the error caused in the final results.

The final procedure and duplicate samples of the chocolate used at Walter Baker Laboratory were submitted to the Central Laboratories of the General Foods Corporation for collaborative work and evaluation. The results checked closely.

The chocolate used in all these experiments contained 25.7 mg of carbon black per 100 g.

TABLE 1

<i>Analyst</i>	<i>Amt. of carbon in mg/100 g of choc.</i>
No. 1.—28.8, 30.4, 26.1, 25.1, 30.1, 26.0, 30.1, 26.0, 30.5, 26.7, 26.0, 30.5, 26.0, 30.5, 26.0, 26.7, 25.9, 26.2, 27.0, 26.5, 25.9, 26.2, 25.5, 26.3	
No. 2.—25.0, 27.0, 30.5	

TABLE 2

The average carbon for Analysts No. 1 and No. 2 in Laboratory 1 was 28.2 mg.

Range of Weight—5.5 mg.
Std. Deviation—1.88 mg.

CONCLUSION

This method is satisfactory for the detection and estimation of carbon black in chocolate. The accuracy of this method depends largely upon the complete digestion of the pulp during the acetylation and may be said to be ± 5 mg. if all conditions have been fulfilled.

ACKNOWLEDGMENTS

The writer acknowledges the cooperation of Mr. N. Ishler and Mr. T. P. Finucane of General Foods Central Laboratories, also Miss C. McDermott and Mr. P. J. Downey of the Walter Baker Chocolate and Cocoa Division of General Foods.

A VOLUMETRIC METHOD FOR THE DETERMINATION OF MAGNESIUM*

By L. J. HARDIN and W. H. MACINTIRE (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

It is to be expected that the trend toward guarantee of magnesium contained in fertilizers will result in the necessity for determination of that element in fertilizer control. This development will call for many additional magnesium analyses in the laboratories of manufacturers and state control officials. The factors of time and convenience then will concern all fertilizer chemists.

The conventional gravimetric method (1) for the determination of magnesium is slow. Even when several samples are analyzed simultaneously, the unit time required is large and the final result cannot be obtained conveniently in less than 30 hours of lapsed time. In case a separation from calcium is necessary, a still longer period is required. As the ignition step of the gravimetric procedure, several hours are required to bring the electric furnace to the requisite $1000^{\circ}\text{C}.$, at which it must be held for 1 hour, and to cool and weight the ignited precipitate. Therefore, any time-saving modification in the last stage of the determination would be helpful.

The precipitation of magnesium as magnesium ammonium phosphate has been adopted universally as the analytical standard, and no modification of that step was attempted. There is a volumetric procedure, (2) however, whereby the precipitate of magnesium ammonium phosphate is filtered by gravity, washed with ammonium hydroxide, air-dried to remove ammonia and the filter then is returned to the beaker where it is titrated with 0.1 *N* acid. As an alternative, the filtration is made onto a Gooch crucible where it is washed with ammonium hydroxide and with alcohol. The precipitate is then dissolved from the crucible by means of 0.1 *N* acid and the resultant solution and washings are back titrated. Using either alternative, this procedure is somewhat shorter than the one in which the precipitate is ignited in the furnace, although the drying requires considerable time and the dried precipitate does not undergo dissolution readily in the 0.1 *N* acid.

In the attempts to shorten the time for the magnesium determinations, the final steps of the established gravimetric and volumetric procedures were subjected to four variations. These were (a) filtration by suction on a weighed fritted glass crucible of "G" porosity and drying in an atmosphere of 50 per cent relative humidity (induced by means of 43.5 per cent H_2SO_4 in a desiccator) after which the dried $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ was weighed; (b) procedure as in (a), but dried at $40^{\circ}\text{C}.$ and the MgNH_4PO_4

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D.C., October 11-13, 1948.

·6H₂O weighed; (c) filtration by suction on a weighed fritted crucible of "G" porosity and ignition at 500°C; (d) filtration by suction on a pad of filter paper pulp in a Shimer tube, the precipitate washed five times with 1+19 NH₄OH and then four times with neutral 95 per cent alcohol. The pad that contained the alcohol-washed precipitate then was transferred from the filter tube to the flask or beaker in which the precipitation was made, and disintegrated by means of a jet of water. After the addition of 0.5 ml. of Bromocresol green indicator (0.2 per cent) and a 5 ml. excess of 0.1 N acid (HCl or H₂SO₄) the thoroughly mixed solution-suspension was allowed to stand 15 minutes in an atmosphere free of fumes, and then back titrated with 0.1 N NaOH (0.05 N NaOH may be preferred). Appearance of the initial medium green (pH 4.6) has been found to be the correct end-point. A reference color standard, prepared with the same amount of indicator and filter paper suspension, is helpful in recognizing the end-point. The net titer of 0.1 N acid times 2.015 gives *mgm* of MgO.

ANALYTICAL RESULTS

The results presented in Table 1 were obtained by means of the several modifications in the analysis of aliquots of a 5-gram per liter solution of magnesium nitrate prepared by use of the hexahydrate. The com-

TABLE 1.—*Comparison of results obtained by means of four modified procedures for the determination of magnesium with those obtained by means of the standard gravimetric and volumetric procedures*

MgO VALUES						
ADDED (a)	GRAVI-METRIC (b)	VOLU-METRIC (c)	METHOD I (d)	METHOD II (e)	METHOD III (f)	METHOD IV (g)
<i>mgms</i>	<i>mgms</i>	<i>mgms</i>	<i>mgms</i>	<i>mgms</i>	<i>mgms</i>	<i>mgms</i>
19.63	19.99	19.60	19.71	19.14	20.09	19.92
19.63	19.70	19.60	20.58	18.53	20.09	19.80
19.63	20.27	—	19.35(h)	19.17	20.38	19.80
19.63	—	—	—	—	19.80	19.80
19.63	—	—	—	—	19.29	91.90(i)
19.63	—	—	—	—	—	20.00(i)
Average	19.99	19.60	19.88	18.94	19.93	19.87
Time required (j)	7 hrs.	3 hrs.	25 hrs.	25 hrs.	3 hrs.	30 min.

(a) Computed from formula $\frac{1}{2} \text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$.

(b) Precipitated $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ was filtered by gravity and ignited 1 hr. at 1000°C.

(c) The precipitated $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ was filtered on paper, dried and titrated with 0.1 N acid, using bromocresol green indicator.

(d) Dried 24 hrs. over H₂SO₄ at 50% relative humidity (fritted crucible)

(e) Dried 24 hrs. at 40°C. (fritted crucible).

(f) The precipitated $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ was filtered on fritted crucible and ignited 1 hr. at 500°C.

(g) The precipitated $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ was filtered on Shimer filter with suction, washed with 1+19 NH₄OH, then with alcohol, and titrated with 0.1 N acid, using bromocresol green.

(h) The precipitate was exposed 72 hours

(i) The determination was made after single precipitation

(j) For completion of determination after the final filtration of magnesium ammonium phosphate.

parisons given in Table 2 were obtained when the proposed procedure was used in the analysis of acid-digested charges of magnesium-fortified fertilizer mixtures that had been analyzed by the gravimetric method. The conventional double-precipitations were used, except as noted.

DISCUSSION

The findings by the conventional gravimetric method were slightly higher than the theoretical values. Similar values were obtained through

TABLE 2.—*Magnesium content of fertilizer mixtures as determined by the proposed volumetric procedure*

	MgO VALUES					
	1		2		3	
	<i>mgms</i>	<i>per cent</i>	<i>mgms</i>	<i>per cent</i>	<i>mgms</i>	<i>per cent</i>
Added in aliquot (a)	8.68	21.70	9.00	9.00	4.81	4.81
Determined, gravimetrically (b)	8.68	21.70	9.00	9.00	9.62	4.82
Determined, volumetrically (c)	8.60	21.50	8.90 9.20	8.90 9.20		
Added in aliquot (a)	21.70	21.70	18.00	9.00	9.62	4.81
Determined, volumetrically (c)	21.60 21.50	21.60 21.50	17.70 —	8.85 —	9.40 9.00 9.50 9.60 9.40 9.30(d) 9.60(d) 9.30(d)	4.70 4.50 4.75 4.80 4.70 4.65(d) 4.80(d) 4.65(d)

(a) Calculated from gravimetric values.

(b) Average of check determinations.

(c) Final filtration was made on Shimer suction filter; precipitate was washed 5 times with 1+19 NH_4OH , then 4 times with small portions of 95% ethyl alcohol.

(d) Same as (c) with the exception that 1+9 methyl-ethyl alcohol corresponding to "Formula 30" was used instead of the 95% ethyl alcohol.

the use of the fritted crucibles and ignition at 500°C .; care must be used, however, to avoid breakage of the crucibles through sudden cooling.

The results obtained by means of the conventional volumetric procedure were in agreement with the theoretical values, when the filter paper and precipitates were air-dried to eliminate ammonia prior to the titration. When compared to the usual ignition, however, this volumetric procedure did not effect appreciable saving of time and the air-dried precipitate did not dissolve readily in 0.1 *N* acid.

The precipitates that were filtered on a fritted crucible and dried in an atmosphere of 50 per cent relative humidity did not reach constant weight in 24 hours. The period of exposure then was extended to 72 hours, after which the weights were virtually constant, and the findings were in satisfactory agreement with the established values. This procedure, however, did not afford the desired rapidity.

The lowest values were those obtained when the precipitate was dried 24 hours at 40°C. Since constant weight was not obtained in briefer periods of drying, no time advantage was obtained through the use of this procedure.

Only one-half hour was required for the entire operation of filtration of the precipitated $\text{MgNH}_4 \cdot 6\text{H}_2\text{O}$ on a Shimer filter, washing with 1+19 NH_4OH and then with 95 per cent alcohol, return to the precipitation flask or beaker, period of standing and titration. The resultant values were in agreement with the theoretical values and with those obtained by the gravimetric procedure. Repeated determinations gave satisfactory checks. Since the results through single precipitations also were in close agreement with established values, it appears that double precipitation may be omitted, unless unusual accuracy is required.

Filtration upon a Shimer filter and titration of the alcohol-washed, moist precipitate is deemed preferable to either the conventional gravimetric or the volumetric method. When only a few determinations are to be made at a time, the gravimetric method is particularly inconvenient and high in unit time requirement. The step of gravity filtration is slow, an electric furnace is required and from 5 to 6 hours is necessary to attain 1000°C. requisite for a 1-hour ignition, whereas additional time is required for the cooling and the weighing of the crucibles.

In contrast, the proposed rapid procedure of filtration through a pad of pulped filter paper in the Shimer filter and titration of the alcohol-washed precipitate is equally adaptable to a single analysis and to the multiple routine determinations that may become necessary in case magnesium becomes designated as a guaranteed element in fertilizers. Satisfactory results were obtained when the 1+9 methyl-ethyl mixture was used in lieu of 95% ethyl alcohol. This mixture corresponds to the commercial, tax-free, denatured product, "Formula 30."

SUMMARY AND CONCLUSIONS

The conventional steps for the analytical preparation, purification and precipitation of magnesium as magnesium ammonium phosphate as prescribed in the "official" method (1) were used in the present comparison between four modified techniques and the conventional gravimetric and volumetric procedures. The proposed modifications in technique relate solely to the treatment of the precipitate of magnesium ammonium phosphate.

When dried at 40°C., the $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ apparently underwent slight dehydration, whereas complete drying was not effected through a 24-hour exposure in an atmosphere of 50 per cent relative humidity. The time required for desiccation was inadmissible in both cases.

Filtration through a fritted crucible and subsequent ignition of the precipitate at 500°C. was time saving, in comparison with the gravity

filtration and ignition in the electric furnace. However, because of the high breakage of crucibles, this technique is not deemed practical.

The proposed volumetric procedure prescribes filtration of the magnesium ammonium phosphate precipitate upon a pad of filter paper pulp on a Shimer filter, washing the beaker and the pad with dilute NH_4OH and then with either 95 per cent neutral alcohol or reagent "Formula 30." After the washed pad is returned to the beaker and dispersed by means of a fine jet of water, the magnesium content is determined by dissolution of the precipitate in a slight excess of 0.1 *N* acid and back titration with 0.1 *N* NaOH against bromocresol green indicator. When several samples are run concurrently, the 30-minute requirement per determination can be decreased to as little as 10 minutes.

The results obtained through the use of the proposed procedure were in agreement with those obtained by means of the conventional gravimetric determination.

REFERENCES

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- (2) HILLEBRAND, W. E., and LUMDELL, G. E. F., 1929—"Applied Inorganic Analysis" (Page 515), John Wiley & Sons, New York.

THE EVALUATION OF ROTENONE IN DERRIS ELLIPTICA ON THE BASIS OF TOTAL CHLOROFORM EXTRACTIVES

By CALEB PAGAN and DAVID G. WHITE¹

The use of substitutes for rotenone in the past 5 years has not been entirely satisfactory for the control of many insect pests according to the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture (2). It is predicted that rotenone may be in greater demand than heretofore. The selection of high-yielding plants is often a problem because of the rather complicated analysis required to determine rotenone content. A simpler method is to determine the content of rotenone plus rotenoids colorimetrically; this value is closely correlated with the actual rotenone content (1).

The present paper deals with a critical evaluation of data from three experiments with respect to rotenone content, rotenone plus rotenoids, and total chloroform extractives of roots from different varieties of *Derris elliptica* (Wall.) Benth. and of *Lonchocarpus utilis* A. C. Smith. In all cases, the roots were air-dried to constant weight and then ground in a Wiley mill through a 0.5 millimeter screen. Rotenone analyses were

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made according to the official method.² Rotenone plus rotenoids were determined colorimetrically as described by Jones (1). The total chloroform extractives were determined by evaporating 10-milliliter aliquots of chloroform extracts, used in rotenone determinations, on a steam bath followed by drying in an oven at 105°C. to constant weight. Determinations of the total extractives required less equipment and time than either of the other tests.

The data were expressed on a percentage basis and the ratios of rotenone to rotenone plus rotenoids, and of rotenone to total chloroform extractives, were calculated. The ratios were analyzed statistically to establish the standard deviations from the means and the data are summarized in Table 1.

TABLE 1.—The ratios and standard deviations of rotenone to rotenone plus rotenoids and to total extractives in roots of *Derris elliptica*, from three experiments

PLANTS REPRESENTED— VARIETIES AND CLONES	AGE AT HARVEST	RANGE IN ROTENONE	RATIO OF ROTENONE TO ROTENONE PLUS ROTENOIDS		RATIO OF ROTENONE TO TOTAL EXTRACTIVES	
			MEAN	STANDARD DEVIATION ⁴	MEAN	STANDARD DEVIATION ⁴
<i>Varietal experiment</i> ¹	<i>Months</i>	<i>per cent</i>				
Sarawak Creeping	25-29	3.6-5.8	0.374	0.031	0.340	0.030
St. Croix	25-29	1.2-2.0	.389	.025	.333	.023
Rto Piedras Changi III	25-29	3.3-4.2	.552	.023	.479	.021
All 3 varieties	25-29	1.2-5.8	.438	.048	.384	.106
<i>Clonal experiment</i> ²						
MG Changi III	36	6.2-8.7	.489	.034	.436	.016
<i>Harvesting experiment</i> ³						
Sarawak Creeping	14	3.5-6.6	.344	.027	.286	.024
Sarawak Creeping	20	3.2-6.3	.389	.024	.341	.021
Sarawak Creeping	26	3.4-7.4	.403	.029	.361	.019
Sarawak Creeping	32	4.0-6.3	.366	.024	.332	.018
All 4 ages at harvest	14-32	3.2-7.4	.375	.036	.330	.044

¹ Reported by Jones, Merriam A., and Pagan, Caleb. A comparison of three varieties of *Derris elliptica*. *Trop. Agr.* 23(4): 76-80, 1946.

² Reported by Jones, Merriam A., White, David G., and Pagan, Caleb. Evaluation of some clones of *Derris elliptica*. *Trop. Agr.* 23(5): 89-93, 1946.

³ Reported by White, David G., Pagan, Caleb, and Jones, Merriam A. Production of *Derris elliptica* in relation to type of cutting and age at harvest. *J. Agr. Research* (In press).

⁴ Standard deviations were calculated using the following formula:

$$S.D. = \sqrt{\text{variance} \left(\frac{y}{x} \right)} = \sqrt{\frac{\bar{y}^2}{\bar{x}^2} \left[\frac{\text{Var. } \bar{y}}{\bar{y}^2} + \frac{\text{Var. } \bar{x}}{\bar{x}^2} - \frac{2 \text{ Covar. } \bar{x}\bar{y}}{\bar{x}\bar{y}} \right]}$$

Where y = individual rotenone content and x = individual rotenone + rotenoids content (or total extractives as the case may be); and \bar{x} = mean of x and \bar{y} = mean of y .

The rotenone content of the roots in the three experiments ranged from 1.2 to 8.7 per cent, total extractives from 3.8 to 21.4 per cent, and rotenone plus rotenoids from 3.3 to 17.0 per cent.

It is of interest to note the good agreement between the mean ratios of Sarawak Creeping in the varietal and harvesting experiments and between the high yielding MG Changi No. 3 clones and the low yielding Rfo Piedras clones. If we consider the case of same clonal material of one variety, the agreement is even closer. For example, cuttings from the MG Changi No. 3 clones were propagated in the field and at the end of 2 years the roots of 32 plants were dug and analyses made for rotenone, total chloroform extractives, and rotenone plus rotenoids. The mean ratio of rotenone to total extractives was found to be 0.437 as compared with 0.436 for the original material.

These results indicate that the ratio of rotenone to rotenone plus rotenoids, or to the total chloroform extractives, is reasonably constant among varieties, although the least deviation occurred within a variety. Within the Sarawak Creeping variety there were small deviations from the mean ratios of roots harvested at 14 to 32 months but for all practical purposes the ratios will hold with reasonable accuracy between these age limits.

Samples of roots of *Lonchocarpus utilis* selected at random from plants grown in Peru were similarly analyzed.* The rotenone content of these plants ranged from 2.3 to 6.6 per cent. In 49 sample plants the mean ratio of rotenone to rotenone plus rotenoids was found to be 0.447 ± 0.039 , and the mean ratio of rotenone to total extractives was found to be 0.364 ± 0.024 .

Under uniform field conditions, determination of rotenone for each of a large number of samples may not be necessary. Duplicate analyses of rotenone in a composite sample should be made, together with total chloroform extractives, and a ratio established. The rotenone content of individual samples can then be based upon total chloroform extractives. Determination of total chloroform extractives in each sample appears to be the easiest and quickest method of evaluating large numbers of samples with reasonable accuracy.

SUMMARY

(1) A comparison has been made of the ratios of rotenone to total chloroform extractives, and of rotenone to red color value, for roots of *Derris elliptica* plants.

(2) A simple and rapid method of estimating the rotenone content of roots of *Derris elliptica* based on total chloroform extractives, is presented.

* Plants collected by E. C. Higbee, Office of Foreign Agricultural Relations, United States Department of Agriculture.

(3) Less extensive data are presented for *Lonchocarpus utilis* which show that total chloroform extractives may give a good approximation of the rotenone content of these plants.

LITERATURE CITED

- (1) JONES, MERRIAM A., 1945. "The application of a modified red-color test for rotenone and related compounds to *Derris* and *Lonchocarpus*." *This Journal*, 28, 352-359 (1945)
- (2) ROARK, R. C. 1946. Abstracts of recent patents relating to rotenone. E-706 (Processed).

BOOK REVIEW

Standard Methods for the Examination of Dairy Products, Ninth Edition. Published by the American Public Health Association, 1790 Broadway, New York City 19, N. Y. (1948). 6×9½ inches, 373 pages, index, 37 figures, cloth. Price \$4.00.

The manual contains analytical procedures to guide regulatory agencies charged with determining and maintaining the healthfulness of dairy products. It is of equal value to industrial workers desiring to have their products conform to standards of wholesomeness.

The methods deal chiefly with sanitary (bacteriological) determinations, pages 1–221 and 336–343. To satisfy additional needs in public health laboratories, the Association has been granted permission to include certain chemical procedures and filth methods for dairy products as recognized in *Official and Tentative Methods of Analysis*, Sixth Edition, by the Association of Official Agricultural Chemists; and certain vitamin assay procedures as recognized in the *United States Pharmacopoeia*, Twelfth Revision, including the First Bound Supplement thereto, by the Board of Trustees of the United States Pharmacopoeial Convention.

The Ninth Edition is characterized distinctively by an orderly separation in Chapter 1 of directions for guidance of administrative officials. Succeeding chapters outline aseptic sampling procedures and directions for the microbiological examination of fluid milk and cream, butter, cheese, frozen desserts and the ingredients therein; for sterility tests to be applied to dairy equipment; for residual phosphatase in heat-treated products; for vitamin assays; for sediment and extraneous matter; and for certain chemical determinations. Assembled in the last chapter are certain "Screening Methods," the use of which will provide administrators with more continuous information about the sanitary character and the chemical composition of certain dairy products than could be obtained with identical funds and personnel using the more refined, time-consuming methods. Obviously, the results of tests obtained by use of Screening Methods are not regarded as satisfactory in case litigation is required.

The new format makes the manual distinctively helpful to laboratory workers also. This has been accomplished by maintaining uniformity of arrangement and style, by using a system of simple cross-references, and by improving the index. While there has been some sacrifice of historical matter, bibliographical references supply abundant data for the critical investigator or administrator.

A. H. ROBERTSON

FIRST DAY

MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

By J. W. SALE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

At this writing, nine Associate Referee reports and one note have been submitted on alcoholic beverages in support of about 20 recommendations dealing with deletions of methods, revision and adoption of other methods, and plans for future work. These reports speak for themselves and the writer offers comment on only one of them, that is, the one dealing with the determination of methanol. As Associate Referee, Dr. Guymon was appointed to investigate both A.O.A.C. procedures 16.25 to 16.28 and 39.161 to 39.162 for methanol in distilled liquors and in drugs respectively, with a view to bringing about conformity in these procedures, so far as possible. A start has been made this year as reported by Dr. Guymon, with respect to one of the procedures, namely, 16.25, and it is hoped that next year 39.162 can be studied and brought into harmony with 16.25. Specifically, we do not question the part of 39.162 which provides for the removal of esters, etc., with petroleum benzene. That step should no doubt be retained in the drug method. Nor do we question the use of a series of color standards instead of measuring the color with a neutral wedge photometer or spectrophotometer. The procedure that is questioned in that method is the use of 5 milliliters of distillate containing 0.25 milliliter of total alcohols for oxidation with 2 ml of 3 per cent potassium permanganate solution. This amount of potassium permanganate is not sufficient to insure oxidation of all of the methyl alcohol in the presence of such an excess of ethyl alcohol. 16.25 corrects this defect. In this latter method, only 0.25 ml of a 22 per cent solution of total alcohols is used for oxidation, the actual amount of total alcohols being only 0.055 ml, as compared with 0.25 ml in 39.162. However, there is a possibility that the same proportion of methanol will be oxidized in the standards as in the sample, even though the total alcohols are not completely oxidized, due to insufficiency of potassium permanganate. This is one of the important points to investigate next year.

In 1945 Beyer and Reeves published the results of an investigation of the immersion refractometer method, 16.29, for methanol in distilled liquors (*This Journal*, 28, 800, 1945). Among other things they found that if the methanol content is less than about 2 per cent in the sample prepared for the refractometer reading, that method is not to be recommended. Also, the authors found that the scale readings for methanol did not agree with all of the corresponding specific gravities in the table

in 16.30. Method 16.29 should be revised accordingly, even though the discrepancies are very small, and the writer is making a recommendation to that effect.

For the convenience of the Associate Referees, who in the course of their duties arrange to test, revise, and recommend change of status of our tentative A. O. A. C. methods, I am listing below the section numbers of such tentative methods for alcoholic beverages, including this year's recommendations, as well as those of 1945, 1946, and 1947. They are as follows:

Beer (Chapter 14.)

.16, .21 to .25 incl., .28 to .31 incl., .33, .34, .36, .38. *This Journal*, 30, 67 (1947).

Malt (Chapter 14.)

.42, .43, .44, .46, .47, .48, .57, .58, .59, .91, .94, .100, .101.

Yeast (Chapter 14.)

.112 to .115, incl.

Spent Grains (Chapter 14.)

.116 to .124, incl.

Wines (Chapter 15.)

.1, .25, .26, .30, .36, .37.

Spirits (Chapter 16.)

.1, .20, .21, .25 to .28 incl., .35, .36, .37. *This Journal*, 31, 183 (1948).

Artificial Colors (Chapter 16.)

.38, .40.

Tannin (Chapter 16.)

.42, .43

Cordials and Liqueurs (Chapter 16.)

.44 to .67, incl., .70, .73 (See *This Journal*, 31, 183 (1948) under "Distilled Liquors").

The fifth edition of the Book of Methods provided for the use of the immersion refractometer for checking the per cent alcohol in all three chapters (Malt Beverages, Wines, Distilled Liquors) and the procedure was classed as official. In the sixth edition the procedure was retained without change in the chapter on Wines, but was not included in the chapters on Malt Beverages and Distilled Liquors. The use of the immersion refractometer facilitates analysis when a number of samples are to be run and the appointment of an Associate Referee is recommended to consider the advisability of including this procedure in Chapters 14 and 16.

All of the recommendations on alcoholic beverages are appended.

RECOMMENDATIONS*

Malt Beverages, Brewing Materials, and Allied Products:

It is recommended—

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 59 (1949).

(1) That study of methods for determination of essential oil and resins in hops be continued.

(2) That the dye color method for the estimation of color in wort and beer, described in the Associate Referee's report for 1948, be adopted as official, (first action).

(3) That the tentative method for color in beer and wort, *This Journal*, 30, 68 (1947), be dropped.

(4) That work on photometric beer color evaluations be continued.

(5) That study of beer turbidity methods be continued.

(6) That the tentative method 14.112-14.115 for total solids in yeast be adopted as official, first action, including the revision of 14.114, Preparation of sample, for total solids as described in the report of the Associate Referee; and that the description of preparation of sample, 14.114, for total solids in yeast, be revised as described in the report of the Associate Referee on Yeast for 1948.

(7) That the Milos test for caramel, 14.35, be deleted, final action.

(8) That the Mathers test for caramel, *This Journal*, 31, 76 (1948), be studied collaboratively with respect to its application to beer.

(9) That the study of carbon dioxide in beer be continued.

(10) That the direct (non-ashing) orthophenanthroline method, described in Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, pages 32 and 37, for the determination of iron in beer, be studied further (a) with a view to eliminating the use of the reducing agent, hydroxylamine hydrochloride, and (b) use of crystalline ferrous ammonium sulfate in place of metallic iron for standardization.

(11) That further work on copper be postponed, pending outcome of proposed work by the Referee on metals in foods.

(12) That work be continued on polarographic-spectrographic methods for tin in beer.

Wines:

It is recommended—

(1) That chromatographic studies of wines be continued.

(2) That the official Milos test for caramel (15.38) be deleted, final action.

(3) That the official, first action, Mathers test, *This Journal*, 31, 76 (1948) be adopted as official, final action.

Distilled Liquors:

It is recommended—

(1) That the study of methods of analysis with reference to the aging or maturing of whiskey in laminated (plywood) barrels be continued.

(2) That the Mathers test for caramel, *This Journal*, 31, 76 (1948), be adopted as official, final action, for distilled liquors, and that it be studied collaboratively, with respect to its application to cordials and liqueurs, before adoption as final action.

(3) That the official modified Marsh test and the official Milos test, 16.39 and 16.41 for caramel, be deleted, final action.

(4) That the study of colorimetric methods for fusel oil be continued.

(5) That the study of the method (16.22 and 16.23) for fusel oil be continued.

(6) That the official method, 16.29, for methanol in distilled liquors by the immersion refractometer method be studied in the light of the findings of Beyer and Reeves, *This Journal*, 28, 800 (1945).

(7) That an Associate Referee be appointed to determine if the immersion refractometer method for alcohol in 15.4(c) should be incorporated in Chapters 14 and 16.

(8) That the rapid method for proof of distilled spirits, as recommended in the report of the Associate Referee on obscuration test for proof in distilled spirits, be adopted as tentative.

(9) That study be continued of the official modified Denigès method for methanol, 16.25, and the tentative method for methanol in 39.161 and 39.162, to bring about uniformity in these procedures so far as possible.

Cordials and Liqueurs:

It is recommended—

(1) That section "16.45 Specific Gravity, see 14.3" be changed to read "16.45 Specific Gravity, proceed as under 16.2."

(2) That a collaborative study be made of methods for caramel in cordials and liqueurs.

(3) That a collaborative study be made of the two tentative methods for total solids, *i.e.*, 16.51 (a) From sp. gr. of dealcoholized sample and 16.51 (b) By evaporation.

(4) That a collaborative study be made of the tentative method for total acidity, 16.62.

REPORT ON SOLIDS IN YEAST

By ROBERT I. TENNEY (Wahl-Henius Institute, Chicago, Ill),
Associate Referee

The criticism which has been directed to method 14.115 has been largely that agreement could not be obtained between laboratories in different cities, although it could be obtained between several analysts working with the same sample at the same time. Studies recently completed¹ by the American Society of Brewing Chemists were designed to test this method upon a carefully controlled sample and to determine whether the differences were due to the method or to fermentive changes within the sample.

¹ R. I. Tenney, *et al.* Proceedings, Annual Meeting, A.S.B.C., 1948.

Nine laboratories participated in the work and compared results obtained upon each of three different days—all working with a split sample of a yeast which had been autoclaved to destroy all enzymatic action. They also compared the total solids content of various local yeasts over a similar period, but permitted autolysis and other fermentation changes to occur.

Changes in the samples were found to occur which could result in a difference of as much as 4.84 per cent in the total solid content within forty-eight hours and as much as 5.87 per cent in seventy-two hours. However, the standard deviation of all laboratories collaborating was only 0.4 per cent when the sample itself was known not to change.

The conclusion can be drawn, therefore, that method 14.115 is capable of giving close agreement and that the differences noted and criticized are due chiefly to changes within the sample itself.

A few studies of a non-miscible solvent distillation method were also made, but such a method involves the use of equipment not commonly available and is also only as accurate as the sampling. Since the present method does give satisfactory results, there seems no need to include an alternate procedure at this time.

RECOMMENDATIONS*

In view of the above, it is recommended—

(1) That the tentative method 14.112–14.115 for total solids in yeast be adopted as official, first action.

(2) That the present description of sample preparation, 14.114, be revised.

Details of this revision are given in *This Journal*, 32, 82 (1949).

REPORT ON THE OBSCURATION TEST FOR PROOF ON DISTILLED SPIRITS

By GEORGE F. BEYER (Bureau of Internal Revenue, Washington
1, D. C.), *Associate Referee*

In the past it has been the accepted practice in distilleries, rectifying plants, and by others to determine the proof of distilled spirits by the use of a hydrometer, preferably one that had been standardized by the National Bureau of Standards. It has also been known for a long time that any substance lighter or heavier than a mixture of alcohol and water will have some effect on a hydrometer.

Blended spirits (whiskey) may contain substances like blending sherry wine, glycerine, prune juice, etc., all of which are heavier than water and,

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 59 (1949).

therefore, will affect a hydrometer by obscuring the true proof of the spirits.

It is a well-known fact that the most accurate method of determining the true proof (per cent of alcohol) in spirits is the distillation method outlined in the 6th Edition of the *Methods of Analysis*, A.O.A.C. However, for this purpose, considerable apparatus is necessary and also an expert operator. On the other hand, it has been found by hundreds of determinations in nearly all of the branch laboratories as well as in the Washington laboratories that, a procedure of determining the apparent proof of the spirits with a standard hydrometer and the solid contents of the sample according to 16.8, and for every 100 mg. of residue (solids) found per 100 ml. of sample adding 0.4 of 1° proof to the apparent proof, produces very accurate results, with only a minimum amount of simple apparatus which requires no expert to manipulate. The procedure, however, has its limitations; for if the spirits contain 600 or more mg. per 100 ml. this method of correction becomes inaccurate. In other words, the correction factor of 0.4 of 1° proof for every 100 mg. of solids is not sufficiently accurate for use when the residue of the spirits analyzes 600 or more mg. per 100 ml., in which case the distillation method must be used.

In the light of these findings it is recommended* that a method be adopted for the determination of the proof of distilled spirits according to the following procedure.

Determine the apparent proof of the distilled spirits with an accurately standardized hydrometer, preferably one graduated in $\frac{1}{2}$ degrees in proof. Determine the extract (solids) according to Section 16.8, and for every 100 mg extract add 0.4° proof to the apparent proof. NOTE:—If the extract amounts to more than 600 mg, this method does not apply.

REPORT ON FUSEL OIL IN DISTILLED SPIRITS

By GEORGE F. BEYER (Bureau of Internal Revenue, Washington 25, D. C.), *Associate Referee*

Since the last report was made on the shortening of the method for the determination of fusel oil in distilled spirits,¹ further work has been done which shows that still more time may be saved without interfering with the accuracy of the method.

Referring to that place in the determination directing the extraction of the saturated salt solution 4 times with varying amounts of carbon tetrachloride and washing the combined carbon tetrachloride extracts with saturated sodium chloride solution and then with saturated sodium sulfate solution, no time for the period of shaking is mentioned. However, the

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 60 (1949).

¹ Beyer, G. F., *This Journal*, 31, 184 (1948).

accepted practice has been to shake the solution when extracting, and the extract when washing, for 2 minutes. Experiments have shown that one minute shaking accomplishes the same purpose. Further time may be saved by reducing the time of oxidation to 2 hours instead of 4.

Work is being continued to determine how to prevent the losses that appear inherent in this method, and it is also recommended* that a colorimetric method for this determination be investigated. Some work along this line has been done with the use of a spectrophotometer, but not sufficient to make a report at this time.

REPORT ON CHROMATOGRAPHIC ADSORPTION OF WINES

By PETER VALAER (Bureau of Internal Revenue, Washington
25, D. C.), *Associate Referee*

This subject has been listed for a report for several years, but since the original and interesting paper was delivered by George K. Hamil in 1942,¹ no comprehensive report has been made. However, during this interval the original method, its improved procedures and its principles, have been in routine use in some laboratories. During the past two years Paul Simonds and Arthur Etienne have been improving its operation and broadening its scope and application up to the present moment.

At the present time we are found in the center of this work so that a paper on this subject at this time would be incomplete and premature. For this reason it is recommended² that the subject be continued until next meeting, at which time it is hoped that a report or a contributed paper will be ready for publication.

REPORT ON CARAMEL IN ALCOHOLIC BEVERAGES

By PETER VALAER (Bureau of Internal Revenue, Washington
25, D. C.), *Associate Referee*

At the last meeting of the A.O.A.C. the Associate Referee made a report on the above subject³ in which was discussed the collaborative results on a method devised by Alex Mathers, and recommended its adoption.

This method has given good results both before and since its collaborative approval, but its adoption was limited to wine and spirituous liquors. Because it has also been successfully applied to beer and cordials, it has been suggested that collaborative work be done on that class of material during the ensuing year.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 60 (1949).

¹ *This Journal*, 25, 220 (1942).

² For report of Subcommittee D and action of the Association, see *This Journal*, 32, 59 (1949).

³ *This Journal*, 31, 178 (1948).

Before the next meeting in 1949, it is planned to send out samples of beer and cordials, with and without caramel, for collaborative work, to obtain a report and recommendation at the next October meeting.

No report was given on cordials and liqueurs or carbon dioxide in beer.

REPORTS ON HOPS

By D. E. BULLIS (Chemist, Oregon Agricultural Experiment Station, Corvallis, Oregon), *Associate Referee*

No collaborative work has been done on the methods for hop analysis during the past 12 months. However, two points in the methods have been examined in a preliminary way in the Associate Referee's laboratory. Because of a lack of adequate analytical assistance more extensive studies could not be undertaken this year. It is hoped that some collaborative work on these items may be completed before the next annual meeting.

Under 14.86(b), page 173 of the 1945 edition of the *Methods of Analysis*, 18–20 ml. is specified as the proper volume in which to carry out the final precipitation of alpha resin. This laboratory has some evidence that incomplete precipitation of alpha resin may result when the above volume is employed with hops of less than average alpha resin content. During the past season we have had occasion to analyze a number of hybrid samples from the U.S.D.A. hop breeding yard at Corvallis, many of which contain less than 4 per cent alpha resin. In many of these the alpha resin-lead compound precipitates with difficulty and incompletely from a 20 ml. volume. By reducing the volume, precipitation takes place normally and apparently much more completely.

The following table illustrates the variation that has been observed.

TABLE 1.—*Variation in precipitation according to volume*

PRECIPITATING VOLUME	ALPHA RESIN-LEAD PRECIPITATE	ALPHA RESIN
ml	gram	per cent
20	.0322 } .0310 } .0316	1.63 } 1.57 } 1.60
15	.0399 } .0418 } .0409	2.02 } 2.11 } 2.07
12	.0442 } .0449 } .0446	2.23 } 2.27 } 2.25
10	.0451 } .0469 } .0460	2.28 } 2.37 } 2.33
7	.0487 } .0486 } .0487	2.46 } 2.46 } 2.46

In the light of these data it may be that the volume of 20 ml. now specified for the precipitation of alpha resin should be reduced, when dealing with old hops or with low alpha resin content hops such as were many lots of the 1947 crop. Further data on the influence of this factor will be obtained during the coming year.

The second point given some preliminary study concerned the precipitation of alpha resin by using a fixed quantity of lead acetate solution. Details of this modification of the usual procedure for this determination are covered in the last two paragraphs of 14.86(b) on page 173 of the 1945 edition of the official methods.

The hops from which the following data were obtained contained 15.97 per cent soft resins, and the results are typical of the errors which are introduced in the alpha resin determination by too little or too much precipitating reagent.

TABLE 2.—*Variation in precipitation according to quantity of reagent*

LEAD ACETATE SOLUTION	ALPHA RESIN-LEAD SALT	APPARENT ALPHA RESIN
ml	grams	per cent
5.2	.0795	5.02
5.6	.0845	5.34
6.0	.0875	5.53
6.4	.0908	5.74
6.8	.0915	5.78
7.2	.0912	5.76
7.6	.0908	5.74
8.0	.0902	5.70

It will be noted that a considerable error results from 1 ml. deficiency of lead acetate, whereas 1 ml. excess does not cause the results to deviate seriously from the correct value.

It is true that 7 ml. of lead acetate is sufficient for precipitating the alpha resin of most hops of 16–19 per cent soft resin content. However, in routine hop analysis, determination of the soft resin content is not usually completed until after the alpha resin determination is finished. Consequently, the analyst has no way of knowing whether he is dealing with a hop of low, average, or high soft resin content, and so has no information on which to base the amount of lead acetate needed for the alpha resin precipitation.

If a rapid estimation of alpha resin is desired it would seem that reasonable speed combined with greater accuracy could best be obtained by omitting the preliminary titration 14.86(a), and carry out the determination according to the first paragraph under 14.86(b). Paragraphs 2 and 3 of 14.86(b) could then be deleted.

Your Associate Referee expects to have some collaborative results on this point to report next year.

REPORT ON INORGANIC ELEMENTS IN BEER

By G. H. BENDIX (Continental Can Company, Inc., Chicago, Illinois),
Associate Referee

IRON

INTRODUCTION

On the basis of the last report (1) and recommendations made on the determination of iron in beer the direct (non-ashing) orthophenanthroline method, based on Nissen's method, were submitted to collaborative study. This Referee has noted that several of the laboratories using a direct procedure for the routine analysis of iron in beer have worked out their own modifications of the Nissen direct orthophenanthroline procedure. The procedure used in this study is as follows:

REAGENTS

10% Hydroxylamine hydrochloride.—Dissolve 100 g of hydroxylamine hydrochloride in 1000 ml of Fe-free distilled water and store in pyrex glassware.

0.3% Orthophenanthroline.—Dissolve 1.5 g orthophenanthroline in 500 ml of Fe-free distilled water heated to 70°C. Cool and store in pyrex glassware.

Iron-free distilled water.—If necessary, water should be redistilled from pyrex glassware.

Glassware:

Pyrex glassware is used. Clean all equipment used with trisodium phosphate, rinse with hot water, rinse with conc. hydrochloric acid, rinse with tap water, and finally rinse with redistilled or Fe-free distilled water.

Standard iron solution:

Dissolve 0.5000 grams of reagent grade iron free of oxide in 5 ml of 20% HCl plus 1 ml of HNO₃. Cover with watch-glass, heat, and evaporate to dryness; add water and evaporate to dryness again. Take up with conc. HCl, cool, and rinse into 100 ml volumetric flask. This is Solution A. Take 10 ml of Solution A +2 drops of bromine water and make up to 500 ml. This is the working standard Solution B; 1 ml = 0.0001 grams of Fe.

Standardization:

Prepare a series of five standards equivalent to 0.5, 1.0, 1.5, 2.0, and 2.5 p.p.m. of iron in a twenty-five milliliter volume. Treat these samples as outlined below and read per cent transmission at 505 millimicrons, using a 4 or 5 centimeter cell in a suitable spectrophotometer. Plot results on semi-logarithmic paper in the usual manner.

PROCEDURE

Pipet 25 ml portions of test beer into two 125 ml Erlenmeyer flasks. Add 5 ml of 10% hydroxylamine hydrochloride to each, mix well, and allow to stand 30 min. Then to one aliquot add 5 ml of orthophenanthroline reagent and to the second aliquot add 5.0 ml of iron-free distilled water. Mix well and allow to stand for 30 min. Using distilled water set at 100% transmission read the two aliquot samples of beer at wave length 505 millimicrons. A blank must be run on each sample of beer analyzed. The p.p.m. iron in the beer is determined as follows:

The p.p.m. Fe read from curve for beer with phenanthroline, less p.p.m. Fe read from curve for beer blank (without phenanthroline).

TABLE 1.—*Results of collaborative study*

COLLABORATOR	METHOD	IRON P.P.M.					
		SAMPLE A DUPLICATES		SAMPLE B DUPLICATES		SAMPLE C DUPLICATES	
NO.		Ave.		Ave.		Ave.	
1	Nissen This report	0.96		0.08		0.47	
		0.99		0.08		0.47	
		—	0.98	0.09	0.08	—	0.46
2	Nissen This report	1.12		0.18		0.64	
		1.14		0.15		0.63	
		1.13	1.13	0.15	0.16	0.63	0.63
3	Nissen This report	(1)	1.00	(1)	0.12	—	
		(2)	0.97	(2)	0.09	—	
	Average		1.02		0.11		0.55
	Max. Deviation		0.11		0.05		0.09
	Average Deviation		0.05		0.03		0.09
4	Ashed— Orthophenanthroline	1.18		—		0.83	
		1.26		—		0.80	
		1.12	1.19	—	0.44	0.85	0.83
1	Wet ashed Orthophenanthroline	1.15		0.07		0.55	
		1.03	1.09	0.10	0.09	0.67	0.61
			1.14		0.27		0.72
3	Direct Bipyridine	(1)	1.11	(1)	0.07	—	—
		(2)	1.02	(2)	0.09	—	—

(1) Sample turbid and run as received.

(2) Sample filtered and then run.

SAMPLES

Three samples were to be run in duplicate by each collaborator. Sample A consisted of canned beer that had been in storage for some time; therefore, a high iron beer; Sample B was a bottled beer, or low iron beer; and Sample C was made up of equal portions of the A and B beers.

DISCUSSION OF RESULTS

The results of Table 1 indicate that the modified Nissen method used in this study is capable of producing results with an average deviation of 0.10 p.p.m. iron or less in the range of 0 to 1 p.p.m. iron in beer. Ashing procedures tend to give high results even when used with adequate blanks. The 2-2' Bipyridine direct method of Gray and Stone compares favorably with the collaborative method outlined herein.

COLLABORATORS COMMENTS

Collaborator No. 2:

"The iron method is very similar to the one we are using, varying only in the relative volumes of beer and hydroxylamine. We have been using an automatic pipet delivering about 45 ml. and only 2 ml. of hydroxylamine. In early work on the method, it was ascertained that results obtained on freshly opened beer were identical even when hydroxylamine was omitted. Our method of standard preparation is slightly different in that we buffer the solutions with citrate-citric acid at a pH of 3.5. The two standard curves, however, differ only slightly."

Collaborator No. 3:

"In our tests with orthophenanthroline and 2-2' Bipyridine, we found that the use of reducing agents, such as hydroxylamine in the direct method is unnecessary. The elimination of this unnecessary step in the method would shorten the manipulations and get rid of an unneeded reagent."

"The use of metallic iron as a standard seems unnecessarily lengthy, especially as the Nissen Method calls for complete oxidation of the iron to the ferric form. Since the ferrous ion is the one that gives the coloration with the reagent the use of crystalline ferrous ammonium sulfate for preparing the standards is suggested."

COLLABORATORS

1. Thomas Blumer, Continental Can Company, Inc., Chicago, Illinois.
2. O. R. Alexander, American Can Company, Inc., Maywood, Illinois.
3. Philip R. Gray, Wallerstein Laboratories, New York City, New York.
4. B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Missouri.

COPPER

The procedure for the determination of copper in foods has been studied collaboratively by the Referee. The results of this study do not warrant a recommendation of a method for determining copper in beer at this time.

TIN

Since the last report, a spectrographic method for the determination of tin in beer was reported on by O. R. Alexander at the 1948 St. Louis meeting of the American Society of Brewing Chemists. Additional work has been done by the Referee's laboratory on a polarographic procedure, but not sufficient to warrant publication of a method.

The Associate Referee is indebted to W. C. Stammer and his associate, Thomas Blumer, for assisting in the above study and for their helpful comments.

RECOMMENDATIONS*

In view of the material reported in the foregoing study, it is recommended—

- (1) That the modified Nissen method be studied further to eliminate the use of the reducing agent, if possible.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 59 (1949).

(2) That the standardization be made using crystalline ferrous ammonium sulfate versus metallic iron to determine the best procedure.

(3) That the potassium thiocyanate procedure previously adopted as tentative be dropped from the Seventh Edition of *Methods of Analysis*.

(4) That the collaborative work on the determination of copper be postponed pending the outcome of proposed work by the Referee on metals in foods.

(5) That no collaborative work be done on tin in beer until the pending polarographic and reported spectrographic methods can be tried in the same study.

LITERATURE CITED

- (1) BENDIX, G. H., *This Journal*, **31**, 172 (1948).
- (2) NISSEN, B. H., Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists. p. 32 (1946).

REPORT ON COLOR OF WORT AND BEER

By B. H. NISSEN (Anheuser-Busch, Inc., St. Louis, Mo.),
Associate Referee

This report describes the final draft of the Dye Color Reference Solution method for the determination of color of wort and beer, which was originally presented to the Association (*This Journal*, **29**, 287, 1946) as an alternative for the present Lovibond procedure (official). (For details of the method, see *This Journal*, **32**, 81 (1949).)

The new Dye Color method has now become the official method of the American Society of Brewing Chemists, and accepted for publication in the Book of Methods of A.S.B.C. as an alternative method. The Dye Color method has been in practice for several years, and is now in use in some 200 brewery laboratories.

It is practically impossible to secure Lovibond apparatus and glasses, so the alternative method is really the only one available.

In the later report* on Color and Turbidity in beer and wort, comment was made on the stability of several of the Dye Color Reference Solutions. These colors showed little, if any, change when tested at yearly intervals; a number of sets have been sent out to various users, all of whom report favorable results. Again this year we can make the same report and repeat that "This Dye Color Reference method appears fully satisfactory for beer and wort color estimation, and furthermore, that their stability is very good."

As a fully proven method it is recommended again for consideration and for inclusion as official in the A.O.A.C. Book of Methods.

* Nissen, B. H., *This Journal*, **30**, 217 (1947).

A photometric procedure is now being studied which it is hoped will terminate in a definition of Lovibond in terms of optical density, with reference to easily reproducible standards.

REPORT ON MALT BEVERAGES, SIRUPS, AND EXTRACTS, AND BREWING MATERIALS

By STEPHEN LAUFER (Schwarz Laboratories, Inc., New York,
N. Y.), *Associate Referee*

It is well known that for the past fifteen years the A.S.B.C. has co-operated with the A.O.A.C. in developing and testing methods for the analysis of brewing materials and products. Because of the efforts of the members of both Associations, most of these methods, after careful collaborative checking, received the status of "Official, final action."

During the past year the activities of the technical subcommittees of the A.S.B.C. were concerned with a number of projects, some of which are also of interest to the A.O.A.C.

STANDARD METHODS

In place of the annual collaborative analysis of one or two samples of malt, a quarterly check sample service was instituted this year. The malt subcommittee has undertaken the distribution of samples and of the summary of results of analysis on two malts each quarter year. This summary is forwarded promptly to the participating laboratories to permit the frequent comparison of results. Over forty laboratories in the United States, Canada, and Denmark are employing this check sample service. A full report will become available at the end of the calendar year; it will also be submitted at the next annual meetings of the A.S.B.C. and A.O.A.C.

A subcommittee on sampling was organized to conduct collaborative studies in order to evaluate quantitatively the variations involved in sampling, particularly of bulk materials such as grains. A preliminary report may be available next year.

A subcommittee on statistics was also appointed, which will assist the other subcommittees, such as those on malt and sampling, in the statistical evaluation of analytical data.

The subcommittee on color of wort and beer continued its work on the Dye Color Reference solutions. Associate Referee B. H. Nissen will submit this procedure for adoption by the A.O.A.C., as an alternate method for determination of color in wort and beer.¹

The subcommittee on yeast continued its collaborative tests on the

¹ *This Journal*, 32, 59 (1949).

determination of total solids in yeast. The results show good agreement between collaborators, and Associate Referee R. I. Tenney will present his report on the subject.

Collaborative work on determination of carbon dioxide in beer has also been concluded. A final report including the revised procedure is now under consideration by the A.S.B.C., and will be submitted next year for adoption by the A.O.A.C.

NEW METHODS

In the past few years two subcommittees were active on the development of methods for barley examination and for biological examination of yeast. In addition to the regular feed analysis on barley, the collaborative tests were concerned with the development of methods which are of main interest to maltsters and brewers, such as germination, potential extract, and potential amylase content. The biological tests on yeast dealt with microscopical examination of yeast, dead cells, counting of cells with the haemocytometer, etc. If the A.O.A.C. is interested in these methods, arrangements will be made for presentation of reports including procedures and supporting data.

NEW EDITION OF METHODS OF A.S.B.C.

A new edition of these methods is in preparation and will be published soon. In addition to new procedures on barley and biological examination of yeast, the new edition will contain minor editorial changes clarifying the old methods. These changes will also be brought to the attention of the Association of Official Agricultural Chemists.

REPORT ON METHANOL IN DISTILLED SPIRITS

By JAMES F. GUYMON (University of California, Davis, California),
Associate Referee

The reliability of the method for determination of methanol in distilled spirits has been questioned by Morison,¹ who obtained inconsistent results both in preparation of a concentration curve using known standards and when applied to brandy. However, his procedure departed in several respects from the official A.O.A.C. method.² During the oxidation step using potassium permanganate, 5 ml. of 5 per cent alcohols (0.25 ml.) were present, whereas the A.O.A.C. method specifies 0.25 ml. of 22 per cent alcohols (0.055 ml.). Beyer³ found that the intensity of the color produced is decreased as the quantity of ethyl alcohol present is increased above the

¹ *Wines and Vines*, 28, (9) 25-27 (1947).

² *Methods of Analysis*, A.O.A.C. (1945), 16.25.

³ *This Journal*, 22, 151-156 (1939).

quantity specified in the official method. Morison reports using a blue filter with the filter photometer employed, whereas Beyer has also shown that maximum absorption occurs in the region of $580\text{ m}\mu$, indicating the desirability of a yellow filter in order to obtain maximum sensitivity. Furthermore, Morison did not employ a distilling head and fractionating column but chose to distill a volume equal to 75 per cent of the volume of the sample. These departures from the official procedure, together with other uncontrolled factors such as temperature, may have caused the inconsistencies reported. Careful examination of his data would not appear to justify the conclusion made that the method fails to distinguish which aldehydes in a mixture may have been derived from methanol.

Prior to more collaborative study of the method for methanol, it was decided to further investigate the influence of certain factors such as temperature, time of standing and light conditions, the spectral characteristics of the color formed by the modified Schiff reagent, and the efficiency of recovery in the distillation step.

The wave length corresponding to maximum light absorption was found to be 570 to $580\text{ m}\mu$ when the spectral transmittance curves were prepared from readings made upon two concentrations of methanol employing a Beckman spectrophotometer. Subsequent measurements were made with a Model 11, Coleman Universal spectrophotometer at $575\text{ m}\mu$.

The time of standing necessary for maximum color development is dependent upon methanol concentration as well as temperature. Extinction readings ($-\log T$) for both 0.05 and 0.5 per cent methanol in 22 per cent total alcohols at different temperatures after varying periods of standing are shown in Table 1. As the temperature is increased, the color not only forms more rapidly but also develops a greater depth. The higher concentration of methanol resulted not only in a more rapid development of color than the lesser amount when compared at the same temperature, but also led to greater depth of color based upon apparent extinction coefficients. The dependence of both rate of development and depth of

TABLE 1.—*Effect of temperature upon color development*

METHANOL	HOURS OF STANDING	EXTINCTION		
		15°C.	20°C.	25°C.
per cent 0.05	0.5	0.002	0.017	0.031
	1.0	0.023	0.041	0.068
	2.0	0.048	0.100	0.102
	5.5	0.094	0.131	0.135
0.50	0.5	0.234	0.453	0.886
	1.0	0.580	1.000	1.569
	2.0	1.125	1.658	1.959
	5.5	1.699	1.721	1.745

color upon temperature and concentration shows that Beer's law is not followed, as already reported.³

It is recommended* that further study be made of the influence of temperature, methanol concentration, and other physical factors, with a view toward establishing conditions suitable for employment of photo-electric measurements. It is also recommended that further study be made of the efficiency of recovery of methanol by distillation in laboratory columns.

REPORT ON CACAO PRODUCTS

By W. O. WINKLER (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

In the report of the Referee last year, the work outlined for this year included five different ingredients of cacao products, viz., lecithin, lactose, maltose, pectic acid, and cacao ingredient. Work has been reported on three of these subjects this year.

Lecithin.—A collaborative study of the method reported in 1946 was made by the Associate Referee. Results obtained show great improvement over those of the previous year. An average recovery of 92 per cent of the added lecithin was obtained, as compared with 78 per cent in 1946. Considering the nature of the material and the quantity of constituent present, the recoveries this year are approaching sufficient accuracy. The Referee concurs in the recommendation of the Associate Referee that the work be continued for another year.

Lactose in the presence of other reducing sugars—As indicated in the Associate Referee's report on this subject, a fermentation procedure for lactose was developed by chemists at the Walter Baker Chocolate and Cocoa Division of General Foods, with some suggestions by the Referee. Results indicate the methods to be reproducible and quite close to the theoretical. In general, the results are a little high on the samples reported, but as almost all of these samples contained "Frodex" (corn syrup solids), the difference is no doubt due to the small amount of higher sugars in the corn syrup which were unfermented by the yeasts used. In the opinion of the Referee, a correction for these could be made by obtaining the rotation of the solution as well as the copper reducing power, and estimating the corn syrup solids by the difference in rotation as found and that calculated from the apparent lactose found. The Referee's one criticism of the method is the time required for an analysis. Since the sample is incubated for about 5 days with the *Sacchromyces fragilis* fermentation, it is entirely too long as a control method. It is recommended that a study be made of ways of shortening the method by the use of larger quantities of the ferment, or by other suitable means, and that the work be continued.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 60 (1949).

Maltose and cacao.—No report will be made on these materials this year and it is recommended that the work be continued.

Pectic acid.—Collaborative work was done on milk chocolate. A sample of milk chocolate was prepared, containing 15.6 per cent of chocolate liquor, representing 6.97 per cent of fat-free cacao in milk chocolate. To 1986 grams of this material was added 14 grams of cacao shell. The original liquor used in the milk chocolate contained 0.5 per cent of cacao shell, making in all 10.2 per cent of shell in the fat-free cacao present in the final sample. Portions of the sample were sent to several collaborators, with the request that they determine the per cent pectic by both the present tentative method and an alternative method, and to report also the weight of cacao residue found by the two methods.

The alternative method differs from the tentative method in that it involves the use of sodium acetate in place of ammonium oxalate to extract the milk protein from the cacao portion, and the separation of any pectin from the extracted milk protein by precipitation with barium hydroxide. The method also makes use of a continuous glass stirrer during the extraction of pectin from the cacao with ammonium oxalate.

Results received from three collaborators, and those of the Referee, are given in Table 1.

TABLE 1.—*Extraction of pectin*

COLLABORATOR	PECTIC ACID				DRY FAT-FREE RESIDUE FOUND×2			
	TENTATIVE METHOD		ALTERNATIVE METHOD		TENTATIVE METHOD		ALTERNATIVE METHOD	
	<i>per cent</i>		<i>per cent</i>		<i>g</i>		<i>g</i>	
1	0.43	0.42	1.11*	0.376	6.38	6.47	10.77	10.91
2	0.28	0.28	0.33	0.38	6.229	6.465	11.239	11.306
3	0.44		0.245	0.35	5.923	5.842	10.146	10.996
4	0.24		0.69		6.392		10.475	

* Probably contaminated with some milk protein.

A study of the results indicates that the milk protein was not as thoroughly extracted with the sodium acetate as was anticipated, and that some of the protein contaminated the cacao residue and the pectic acid precipitate. The residue showed a great increase in weight and developed into a horny mass on drying. Results by the tentative method were more satisfactory, and the cacao residue found was fairly close to the theoretical.

It is the Referee's opinion that the best features of both methods could be combined for a more rapid and accurate method. A feature which could be incorporated in the tentative method to advantage is the use of a continuous stirring device during the extraction of the pectin with

ammonium oxalate. This would no doubt give a better and more uniform extract.

The following change is proposed in the tentative method:

Neutralize to litmus with NH_4OH (1 + 1) (ca 1 ml), then make slightly acid with acetic acid and add 50 ml of 2% NH_4 oxalate soln. Place power driven glass rod stirrer with a vertical loop at the end in the flask with the shaft thru tube inserted in a No. 10 rubber stopper. Place flask in water bath held at 90–92°C. and stir contents gently and continuously for three hours.

RECOMMENDATIONS*

It is recommended—

- (1) That the method for lecithin in cacao products be further studied.
- (2) That the method for lactose in cacao products, reported this year, be studied to increase the rapidity of the method and to correct for the effect of the presence of higher sugars in corn syrup solids.
- (3) That the study of methods for maltose and cacao ingredient be continued.
- (4) That the tentative method for pectic acid, 19.16, be revised as recommended by the Associate Referee.
- (5) That the method for pectic acid in milk chocolate be further studied.
- (6) That the method of separation of fat, 19.25, when used on milk chocolate, be studied and compared with the method for refractory sample proposed by Ferris. *This Journal*, 31, 728 (1948).

REPORT ON LECITHIN IN CACAO PRODUCTS

By JOHN H. BORNMAN (Food & Drug Administration, Federal Security Agency, Chicago, Ill.), *Associate Referee*

The collaborative results reported by the Associate Referee in 1946, (*This Journal*, 30, 281), showed fairly good agreement among analysts, but the recovery of added lecithin (78%) was considered to be too low. No samples were sent out for collaboration in 1947 because the Associate Referee was not able to improve materially on the per cent recovery, although a fresh sample of lecithin was used and samples were carefully prepared.

Two samples were sent to collaborators this year for the determination of lecithin by the method outlined in the 1946 report referred to above. One sample was a sweet chocolate (A) and the other (B)—a portion of the same with 0.25% pure lecithin added.

Results reported by collaborators are given in the following table.

* For report of Subcommittees D and action of the Association, see *This Journal*, 32, 60 (1949).

SAMPLE A		SAMPLE B		
COLLABORATOR	LECITHIN	LECITHIN	ADDED LECITHIN FOUND	RECOVERY
	<i>per cent</i>	<i>per cent</i>	<i>(B-A)</i>	<i>per cent</i>
1	0.186	0.407	0.221	88.4
	0.186	0.402	0.216	86.4
2	0.200	0.443	0.243	97.2
	0.182	0.443	0.261	104.4
3	0.152	0.439	0.287	114.8
	0.205	0.439	0.234	93.6
4	0.239	0.432	0.193	77.2
	0.230	0.425	0.195	78.0
5	0.151*	0.371*	0.220	88.0
	0.160*	0.382*	0.222	88.8
6	0.202	0.425	0.223	89.2
	0.200	0.421	0.221	88.4
7	0.205	0.455	0.250	100.0

* Corrected for reagent blank.

The Associate Referee believes that, with sufficient experience, an analyst can check his results to within 2 per cent of the value. His results (Collaborator No. 6) represent the maximum and minimum of 5 determinations on A and 4 on B, obtained on three different dates. The average recovery of all collaborators was 92 per cent. No reason for the low recovery has been found.

Lecithin was determined on an aliquot of a chocolate extract and on an aliquot of a lecithin solution. Equal aliquots of the two solutions were mixed and the lecithin determined on the mixture equalled the sum of the determinations on the separate aliquots, within experimental error (0.221 to 0.224 mg). Thus there is no loss under these conditions; however, when lecithin is incorporated in chocolate it does not appear to be completely recoverable by extraction.

In order to determine whether phosphoric acid is lost in the digestion with larger amounts of fat present, an aliquot of the lecithin solution was digested with 1.13 g. olive oil, free from phosphoric acid, added. No loss was detected.

The advantage of the proposed method is due to the fact that the extraction involves less work than a method whereby a sufficiently large sample of chocolate is extracted to yield enough phosphoric acid for a determination by the phosphomolybdate method. The main difficulty

lies in the application of the molybdenum blue method, which, however, is necessary for the determination of the small amounts of phosphoric acid involved. The fact that recovery is about 90 per cent is not a great disadvantage when it is considered that accurate estimation of the added lecithin depends on a knowledge of the amount naturally present, which Winkler & Sale (*This Journal*, 14, 543(1931)) found to vary from 0.25 to 0.46 per cent in different varieties of cacao.

SUMMARY

Collaborative results reported in 1946 showed an average recovery of only 78 per cent of the lecithin which was added. No samples were sent out in 1947 because the Associate Referee was unable to improve recovery materially. This year the average recovery of 7 collaborators was 92 per cent. No adverse comments were reported.

The proposed method is considered advantageous because of the ease with which an extract is obtained for determination of phosphoric acid.

Digestion of lecithin in presence of large amount of fat does not result in loss of phosphoric acid. Results obtained on a mixture of a lecithin solution with a chocolate extract are the same as the sum of equivalent aliquots run separately. When lecithin is incorporated in chocolate there is an unexplained loss of lecithin. Because of the variation in lecithin naturally present in cacao, a loss of 10 per cent in recovery of added lecithin is not considered of great importance.

It is recommended* that work on lecithin be continued.

ACKNOWLEDGMENT

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- H. C. Van Dame, Cincinnati District
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- W. Horwitz, Minneapolis District
- F. E. Yarnall, Kansas City District
- M. L. Dow, St. Louis District
- G. McClellan, New Orleans District.

REPORT ON LACTOSE IN CHOCOLATE

By MARY E. HANLON, F. V. KENNEY, JR.† and D. G. MITCHELL
(Associate Referee), (Walter Baker Chocolate and Cocoa Division, General
Foods Corporation, Dorchester, Mass.)

SUMMARY

A method suggested by W. O. Winkler of the Food and Drug Administration for the determination of lactose in milk chocolate in the presence of other reducing sugars has been developed. The method is considered

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 60 (1949).

† Mr. Kenney's present address is White Rock Beverage Corporation, Brooklyn, New York.

of value in view of the reproducibility of results and the moderate error from the theoretical quantity added to the milk chocolate.

INTRODUCTION

The determination of lactose in milk chocolate containing other reducing sugars by the regular methods of lactose analysis has been impossible because of the interference of other materials. Such reducing sugars as dextrose and maltose when present with lactose give similar reactions in such methods as the Munson and Walker, Lane-Eynon, Soxhlet, Defren, and others. (1) The determination of lactose in the presence of sucrose has presented no problem and several methods have been established.

Magraw, Copeland, and Sievert (2) developed an improvement on the method of Magraw and Sievert (3) for the determination of lactose in the presence of other carbohydrates. This method is based on an enzymatic treatment using animal diastase, invertase-melibiose scales, and bakers' yeast for destruction of interfering materials. The lactose is then determined by the Munson and Walker method. This procedure has been tried unsuccessfully on milk chocolate. When tried on milk chocolate containing corn syrup solids, quantities of lactose in excess of the known amount were found. It is possible that the enzymes used are not effective in the presence of chocolate as they were intended for complex mixtures of mixed feeds.

A similar method for the determination of lactose in milk chocolate has been described by F. Th. Van Voorst (4). This method was found unsuitable when other reducing sugars are present.

The method presented below is that suggested by W. O. Winkler of the Food and Drug Administration. The use of *Saccharomyces fragilis*¹ was not originally suggested but was later recommended by Mr. Winkler when the yeasts were not found to be sufficient.

PROCEDURE

Place 12 grams of chocolate sample (accurately weighed) in a 250 ml centrifuge bottle; extract twice by shaking with 100 ml portions of petroleum ether, centrifuging and decanting supernatant liquid each time. Allow ether to evaporate by placing the bottle in a warm place (about 40–45°C.)

Add 60–70 ml of water at 60°C. to the residue in the bottle, stopper, and shake well for 3 min. to dissolve sugars and disperse residue.

Add one g of dry brewers' yeast. Add 6–7 g of Hyflo supercel and a suspension of 6–7 g of washed bakers' yeast in water to centrifuge bottle and mix. Allow to stand at temp. of ca 35°C. for 4 hours, rotating flask occasionally. Conduct a blank using 4 g of sucrose and 1 g of dextrose. Filter the mixture on an 11 cm Büchner funnel with suction, using an 11 cm CS & S blue ribbon filter paper and a suction flask. Wash centrifuge bottle and filter several times with about 15 ml portions of water. Remove the paper and residue from the funnel, strip off paper and place residue in a 600 ml beaker. Add about 40 ml of water and triturate to thoroly disperse the material. Return paper to funnel and decant mixture in the beaker again to the

¹ The *Saccharomyces fragilis* was obtained from American Type Culture Collection, Georgetown University, School of Medicine, Washington, D. C.

funnel, rinse beaker, and suck dry. Transfer combined filtrate to 250 ml volumetric flask. Add 1 ml of 10% tannic acid, and wash down with a little water, and add 2 or 3 ml of basic lead acetate slowly with rotation to clarify the soln. Allow to settle and test with 1 drop of the clarifying soln for complete precipitation. Make to the 250 ml mark at 20°C., mix, and filter. Discard about the first 15 ml of the filtrate. Add a slight excess of dry $\text{Na}_2\text{C}_2\text{O}_4$ to delead the filtrate by adding salt in small amounts with stirring and, after settling, test for complete precipitation with a few crystals of oxalate. Filter off precipitate, discard the first 15 ml.

Inoculate the filtrate with a loopful of *Saccharomyces fragilis*, plug with sterile cotton, and incubate for five days.

Filter and determine the reducing sugars by the Munson and Walker method of copper reduction (*Methods of Analysis*, A.O.A.C. 1945) on 50 ml of the soln. Obtain the lactose from Table 44.11, 6th Ed. Calculate the total lactose in the sample taken and divide by the weight of the sample to obtain the per cent of lactose.

The *Saccharomyces fragilis* was cultured in an agar medium made up as follows:

Water	—1000 ml
Bacto-peptone—	15 g
Glycerol	— 5 g
Dextrose	— 20 g
Agar	— 20 g

Incubate at 30–35°C.

RESULTS

The samples of milk chocolate used for analysis in these tests were of a normal formulation but were especially prepared for accuracy of composition. Sample A of composition shown below was prepared using whole milk solids of known lactose content. "Frodex," or commercial corn syrup solids, was added as a source of glucose and maltose.

Sample A

	Per cent
Sucrose	50.5
Lactose	4.42
"Frodex"	4.76

Analyses were made of successive samples of this chocolate over a period of a week according to the procedure described above. Blanks were run in all cases as prescribed in the method.

Analysis of Sample A

Anal. No.	% Lactose
1	4.74
2	4.73
3	4.70
4	4.70
5	4.70
6	4.70
Average	4.71
Theoretical	4.42

Analysis was made of another lot of milk chocolate, Sample B, whose composition was slightly different. The composition of this chocolate was as shown below:

<i>Sample B</i>		
	<i>Added</i>	<i>By Analysis</i>
	<i>Per cent</i>	<i>Per cent</i>
Sucrose	40.0	40.16
Glucose	4.55	—
"Frodex"	4.55	—
Lactose	5.60	5.45

In order to produce an accurate composition and without an interference from other substances, a special sugar feed, Sample C, was compounded from pure substances. The materials were added in approximately the same ratio as would be found in a milk chocolate. Below is shown its composition and the analysis obtained.

<i>Sample C</i>	
Sucrose	4.4 gm.
"Frodex"	0.6 gm.
Lactose	0.6 gm. (theoretical)
Lactose	0.57 gm. (by analysis)

Another test was made, where additional quantities of dextrose and maltose over and above that present in the corn syrup solids were added. The composition of this sample is shown below:

<i>Sample D</i>	
	<i>Per cent</i>
Sucrose	46.00
Dextrose	4.35
"Frodex"	4.35
Maltose	4.35
Lactose	4.03 (theoretical)
Lactose	2.96 (by analysis)

Only about 75 per cent of the lactose was recovered on this sample. It is possible that the higher percentage of reducing sugars was greater than could be reacted with the quantities of reagents used. A recheck of this sample was made using a longer fermentation to determine if a better recovery of lactose could be made. After ten days fermentation, lactose was found to be 4.70 per cent. Further fermentation was impossible because of the interference of bacteria at this time.

This proportion of reducing sugars might be further investigated to determine if the quantity of maltose is a limitation of the method or if the quantity of reagents is insufficient. However, it is felt that this proportion of maltose is greater than would normally be encountered in a sample of chocolate, therefore the results may be more or less of academic interest.

Since the above analyses were all performed by the same analyst, prepared samples, Sample E, were sent to Central Laboratories of General Foods Corporation, Hoboken, N. J. for analysis. The method was prescribed and a sample of *Saccharomyces fragilis* supplied. They used the method previously described with one modification. The sugars were determined by the Lane and Eynon titration rather than the Munson and Walker method, because of their greater familiarity with it.

Sample E

	<i>Per cent</i>
Sucrose	52.00
"Frodex"	5.00
Lactose	5.20

The chocolate sample was run in triplicate. Two blanks were used consisting of one gram of dextrose and four grams of sucrose in each, and two controls were also used consisting of one and two grams of lactose with one gram of dextrose and four grams of sucrose. The results are presented below:

SAMPLE	LACTOSE	ADDED DEXTROSE	SUCROSE	LACTOSE FOUND
				<i>per cent</i>
E	—	—	—	5.75
E	—	—	—	5.76
E	—	—	—	5.82
Blank	—	1	4	—
Blank	—	1	4	—
Control	1	1	4	99.3 (recovery)
Control	2	1	4	98.9 (recovery)

As will be noted, the results are slightly higher than the amount present and the difference is a little greater than that obtained in the Munson-Walker method. Although it is believed there is no difference in the results by the two methods for determining the sugars it is possible that this is the cause of the discrepancy. This analysis has been run and the lactose content determined as 5.53 per cent. In view of the Central Laboratory's result, it is felt that there is a reasonably good check between two analysts using the same method independently.

From the work outlined above it is considered that the method is successful. The analyses indicate that the results are reproducible with a moderate difference from the theoretical value. Samples containing higher sugars were not prepared as it was felt that they would not be found normally and would be only of academic interest.

It is recommended* that the method for lactose be studied collaboratively.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

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- (3) *Ind. Eng. Chem., Anal. Ed.*, 7, 106 (1935).
- (4) *Chemisch Weekblad*, 34, 803 (1937).

No reports were given on malt solids, pectic acid, cacao ingredients, or fat.

The contributed paper entitled "Milk Fat in Milk Chocolate," by L. W. Ferris, was published in *This Journal*, 31, 728 (1948).

REPORT ON FRUITS AND FRUIT PRODUCTS

By R. A. OSBORN (Division of Food,* Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

PRELIMINARY REPORT ON METHODS FOR THE DETERMINATION OF FILL OF CONTAINER OF FROZEN FRUITS

Work is now in progress on methods for the determination of fill of container for the smaller (1 lb.) packages of frozen fruit. It appears that the per cent fill can be obtained by a displacement procedure which is a modification of the tentative procedure of this Association for Ice Cream and Frozen Desserts—weight per unit volume of packaged ice cream (*Methods of Analysis*, A.O.A.C., 6th ed., 22.143). An overflow can filled with deodorized kerosene¹ is placed in a deep freeze cabinet at or near 0°F. and the frozen food, removed from its wrapping, is immersed in the liquid. The overflow of liquid is the measure of the capacity of the frozen food. In order to provide for interstices within the frozen food, use is made of a synthetic latex bag.² The frozen food is placed in the bag which is evacuated and tied before immersion. The work on this project is still in progress and a more complete report may be expected later.

A rapid method for water-insoluble solids and also one for determining the weight of seeds in berry fruit are recommended for adoption.

NOTE ON THE SODIUM COBALTINITRITE REAGENT FOR THE DETERMINATION OF POTASSIUM IN FRUITS AND FRUIT PRODUCTS

Under 26.19, sodium cobaltinitrite solution is used in the determination of potassium. The analyst is directed to prepare and use a control sample of potassium chloride containing 2 mg. of potash per ml. as a check on the suitability of the reagent. It is stated "If recoveries are low, reagent should

* W. B. White, Chief.

¹ Bayol D, manufactured by Standard Oil Co. of New Jersey.

² Cry O Vac bag, manufactured by Dewey and Almy Chemical Co., Cambridge, Mass.

be rejected; if slightly high, blank corrections may be made on the K estimations."

Those who are familiar with the reagent have doubtless observed that different lots may vary in physical properties, particularly in color. The writer has been advised by those engaged in the manufacture of the reagent that it is not a definite chemical compound. Since the analyst is

TABLE 1.—*Comparisons of lots of sodium cobaltinitrite reagent*
(Recoveries using 20 mg K₂O in KCl solution)

FIRM	LOT	K ₂ O RECOVERED	RECOVERY
		<i>Mg</i>	<i>per cent</i>
A	1	20.16	100.8
A	1	20.49	102.4
A	1	20.50	102.5
A	1	20.53	102.6
A	1	20.59	102.9
B	2	20.22	101.1
B	2	20.41	102.0
B	3	19.89	99.5
B	3	19.93	99.7
C	4	19.95	99.8
C	4	19.99	99.9
C	5	21.11	105.5
C	5	21.17	105.8
C	6	19.43	97.2
C	6	19.85	99.2
C	7	20.74	103.7
C	7	20.84	104.2
C	7	20.95	104.7
C	7	21.01	105.0
D	8	19.58	97.9
D	8	19.72	98.6

primarily concerned with its suitability as a reagent in the quantitative determination of potassium, the fact that the reagent does not have a definite chemical composition is immaterial if reasonable recoveries of potash are obtained by its use. The writer has tested samples of the cobaltinitrite reagent manufactured by each of four firms located in the United States. In carrying out the comparisons 10 ml. of a potassium chloride solution was taken, each ml. of which contained an equivalent of 2 mg. of potash.

It will be observed from Table 1 that the tendency is for slightly high rather than low recoveries. In the interpretation of the results of a fruit

analysis, the greater than 100 per cent recovery of potash would lead to a slightly higher estimation of fruit content. Two of the lots examined gave potash recoveries in the neighborhood of 105 per cent. It would be preferable to reject such lots and use a lot which will give recoveries more nearly 100 per cent. It is recommended that the reagent be described merely as sodium cobaltinitrite rather than by a formula, that each lot of the reagent be tested before use, and that lots be rejected which give recoveries of less than 98 per cent or more than 102 per cent with 20 mg amounts of K_2O .

RECOMMENDATIONS*

It is recommended—

(1) That study of methods for determining fruit and sugar content of frozen fruits be continued.

(2) That further collaborative study be made of the method for the electrometric titration of acidity.

(3) That further study be made of methods for separating and determining fruit acids.

(4) That procedures as given by the Associate Referee in his 1948 report, for the rapid determination of water-insoluble solids and for the determination of seeds of berry fruits be adopted as tentative and that the procedures be subjected to collaborative study.

(5) That 26.18(a) be changed to read as follows:

(a) *Sodium cobaltinitrite soln.*—Prepare an aqueous soln containing 2.0 g of sodium cobaltinitrite in each 10 ml, from a previously tested lot of the reagent giving recovery of not less than 98 per cent and not more than 102 per cent with 20 mg amounts of K_2O . Filter soln before use and prepare fresh soln before each set of determinations.

REPORT ON FRUIT AND SUGAR CONTENT OF FROZEN FRUITS

By R. A. OSBORN (Division of Food,† Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In an article by J. Walter Sale entitled "Interpretation of Chemical Analyses of Preserves and Jams," *This Journal*, 21, 502 (1938), much authentic information is given regarding the chemical composition of fruits grown in the United States, and the procedure for determining the ratio of fruit to sugar in preserves is described. The amounts of fruit and sugar (or sugars) in frozen fruit mixtures can be calculated using the procedure

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 63 (1949).

† W. B. White, Chief.

described by Sale. The problem is somewhat less complicated since the mixtures are not concentrated by the application of heat as is the case with jams, jellies, and fruit butters. It is necessary to obtain a representative sample of frozen fruit "A" and subject it to chemical analysis by the well-known procedures of this Association (Methods of Analysis, A.O.A.C. 6th ed., Chapter 26 (1945)) for such constituents as water-insoluble solids, ash, potash, phosphate, and total sugars after inversion; and then by comparison with authentic data (averages of known samples of the fruits analyzed for their content of sugars, water-insoluble solids, ash, potash, etc.) calculate the fruit and sugar contents of the sample "A." This procedure involves much analytical work. If possible, simple and accurate alternate procedures should be developed. In contested court cases it has been suggested that the comparison of the analytical values obtained should be made with the minimum values of the authentic samples rather than the averages. Sale (*loc. cit.*) points out the fallacy of this proposal.

A considerable amount of preliminary work has been carried out in the Food and Drug Administration with frozen fruits, to determine whether the drained weight under carefully controlled conditions, together with the determination of the soluble solids (as sucrose) by refractometer can be employed as a rapid procedure for determining their fruit and sugar content. The results so far obtained are encouraging. However, the problem is not a simple one, since ripeness (softness) of the fruit is a factor which has an effect on the amount of drained fruit left on the screen.

The work on this problem is still in progress and a report at this time would be premature. Accordingly, it is recommended* that the work be continued.

REPORT ON WATER-INSOLUBLE SOLIDS OF FRUITS AND FRUIT PRODUCTS

By R. A. OSBORN (Division of Food,† Food and Drug Administration,
Federal Security Agency, Washington 25, D. C.), *Associate Referee*

PART I. RAPID METHOD

A former report on this subject¹ deals chiefly with a study of the existing procedure for the determination of water-insoluble solids, as published in *Methods of Analysis*, A.O.A.C., 6th ed., 26.7 (1945).

A rapid procedure for the determination of water-insoluble solids of fruits and fruit products has now been developed. Speed is obtained by rapid preparation of the sample through (1) the use of a Waring Blender,

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 63 (1949).

† W. B. White, Chief.

¹ Osborn, R. A., *This Journal*, 30, 260 (1947).

which in two minutes comminutes and intimately mixes samples weighing up to 2 lbs.; (2) by shortening the period of boiling to a few minutes, since the sample is finely divided; (3) by rapid filtration on a loose textured filter paper of greater area (15 cm diameter circle in a 12½ cm diameter Büchner funnel); and (4) by rapid drying of the filter and water insoluble solids (by the use of an instrument which works on the principle of an electric hair dryer in that hot air is forced on the sample and exhausted through a 500-mesh Monel metal filter cloth bottom). Drying can ordinarily be completed in 10–15 minutes, depending on the size of sample taken for analysis and the amount of material to dry. The procedure does not differ materially from the existing longer procedure and results that have been obtained are comparable with it. The rapid procedure should appeal to the ordinary analyst, and it should find application in factories that process fruit products into preserves, fruit butters, fountain fruits, etc., where it is desirable to control the insoluble solids content of the finished products.

WATER-INSOLUBLE SOLIDS (RAPID METHOD)

APPARATUS

Waring Blendor (or other suitable comminuting device).

Balance (sensitive to ± 1 milligram) and weights.

1-liter suction flask (provision for vacuum).

Büchner funnel (Coors 4 5¼" diam.).

Filter paper.—15.0 cm fast; open texture (Whatman #4, or equivalent).

Weighing dishes.—Aluminum or tinned iron 5¼" diam. \times ¾" high, with close-fitting cover (16 mm film holders obtainable from camera stores). (Al. dishes weigh approximately 40 g, tinned iron ca 85–90 g.)

Rapid drying device:

(a) Moisture Teller Model 271 T, manufactured by Harry W. Dietert Co., 9330 Roselawn Ave., Detroit 4, Mich., or

(b) Forced Draft Drying Oven operating at 100°C.

DETERMINATION

Fit a 15 cm circle of filter paper into a 12½ cm Büchner funnel, add ½ of a 7 cm circle of filter paper (to be used to wipe any insoluble solids from Büchner after filtration and washing the sample), wash with boiling water, apply suction, and dry, using drying device (a) or (b). Transfer to weighing dish, cool and weigh in balance using a tare consisting of weighing dish and paper. (Approximate time of drying, 5 min. at 215°F. \pm 5°F.)

Weigh 25 or 50 g of well mixed sample (Waring Blendor) to nearest .01 g, transfer the sample with hot water to a 400 ml beaker, adjust to approximately 200 ml with hot water, stir, and boil gently for a few minutes. Place prepared filter in Büchner, attach to suction flask but do not attach flask to suction line. Pour 50 to 100 ml of boiling water on filter and when a steady flow of water passes thru filter, transfer the sample to the filter, portionwise if necessary. Wash insoluble solids with boiling water and collect approximately 850 to 900 ml of filtrate. (In the washing operation keep the solids from forming a tight mat on the surface by portionwise additions of the boiling water.) Apply suction after concluding the washing operation and aspirate thoroly. Transfer paper and water-insoluble solids and dry as above, us-

ing extra piece of weighed filter paper to complete the transfer, and dry at 215°F. $\pm 5^\circ\text{F}$. (approximately 15 min., depending on amount of water-insoluble solids). After drying, transfer sample to weighing dish, cool in desiccator, and weigh. Weight of water-insoluble solids \div wt. of sample $\times 100 = \%$ water-insoluble solids.

Tabulated data (Table 1) contain comparative results for per cent water-insoluble solids for four commercial preserves analyzed by the rapid procedure as given herein and by the regular published procedure. It will be observed that the results are not materially different.

TABLE 1.—*Comparison of results for water-insoluble solids, regular A.O.A.C. procedure¹ vs. rapid procedure*
All samples 25 grams

SAMPLE NO.	WATER-INSOLUBLE SOLIDS (PER CENT)			
	TYPE OF COMMERCIAL PRESERVE	RAPID PROCEDURE		REGULAR PROCEDURE
		<i>Average</i>		<i>Average</i>
51591 F	Raspberry	2.48		2.47
		2.51		2.58
		2.57		
		2.61	2.54	2.53
62770 F	Peach	0.48		0.49
		0.50	0.49	0.50 0.49
51494 F	Strawberry	1.20		1.13
		1.20	1.20	1.14 1.14
52791 F	Cherry	0.56		0.57
		0.62	0.59	0.59 0.58

¹ *Methods of Analysis, A.O.A.C.*, 6th ed., 26.7 (1945).

PART II. SEED AND NON-SEED WATER-INSOLUBLE COMPONENTS OF BERRY FRUITS

During a study of methods for the determination of water-insoluble solids of fruits and fruit products,¹ a number of samples of commercial berry preserves and a few samples of authentic berry fruits were examined for their content of seeds free from adhering fruit tissue, in addition to the determination of water-insoluble solids. C. L. Hinton and T. Macara in a manuscript entitled "The Composition of Some Jam Fruits and the Determination of the Fruit Content of Jams"² have reported their findings of insoluble solids and seed contents of several berry fruits using analytical procedures which differ from those described here. The procedure for seeds which we employ is simple and rapid.

¹ Osborn, R. A., *This Journal*, 31, 185 (1948).

² *The Analyst*, 65, 540 (1940).

SEEDS IN BERRY FRUITS

Prepare the sample by thoro mixing, using a Waring Blendor. Take 50 g \pm .01 g of the sample, transfer with ca 500 ml of hot water to the mixing chamber of Waring Blendor and mix for 1-2 min. Transfer mixture to a 20-mesh screen and use additional hot water to transfer and wash the bare seeds. (Hot water from the tap is suitable for use in this procedure.) Transfer the seeds on the screen to a 70 mm aluminum dish, previously weighed, with close-fitting cover. (This is readily accomplished by transfer to a 7 cm Whatman #4 circle of filter paper in a Coors 2A Büchner funnel with suction. The paper is previously dried and weighed with the aluminum dish.) Dry at 100°C. in a forced draft oven for 30 min. and weigh. To determine average weight of one seed, count out and weigh separately several 100-unit lots.

TABLE 1.—Commercial preserves with seeds

SAMPLE NO.	WATER INSOLUBLE SOLIDS		BARE SEEDS		INSOLUBLE SOLIDS NOT SEEDS BY DIFFERENCE	NON-SEED OF TOTAL	BARE SEEDS OF TOTAL	AVERAGE WEIGHT OF 1 SEED
	per cent		per cent		per cent	per cent	per cent	Mg
Blackberry								
54164 F	1.38		0.90		0.47	33.8	66.2	3.49
	1.40	1.39	0.94	0.92				
79220 F	2.44		1.81		0.72	28.2	71.8	2.93
	2.64	2.54	1.83	1.82				
55028 F	2.49		1.75		0.80	31.1	68.9	3.24
	2.65	2.57	1.79	1.77				
60470 E	2.93		2.37		0.57	19.4	80.6	2.82
	2.95	2.94	2.38	2.37				
19412 E	3.52		2.60		0.81	23.0	77.0	2.04
			2.81	2.71				
62772 F	3.52		2.81		0.68	17.9	82.1	1.76
	3.53	3.52	2.88	2.84				
54166 F	3.51		2.83		0.77	21.0	79.0	2.98
	3.80	3.66	2.94	2.89				
Average						24.9	75.1	2.75
Logan-(Black) berry								
55128 E	2.70		2.20		0.45	16.7	83.3	1.84
	2.70	2.70	2.30	2.25				
63101 E	3.16		2.47		0.74	22.8	77.2	2.20
	3.34	3.25	2.55	2.51				

TABLE 1.—*Continued*

SAMPLE NO.	WATER INSOLUBLE SOLIDS	BARE SEEDS	INSOLUBLE SOLIDS NOT SEEDS BY DIFFERENCE	NON-SEED OF TOTAL	BARE SEEDS OF TOTAL	AVERAGE WEIGHT OF 1 SEED
	per cent	per cent	per cent	per cent	per cent	Mg
<i>Red Raspberry</i>						
54158 F	2.02	1.55				
	2.12 2.07	1.58 1.56	0.51	24.6	75.4	1.33
62774 F	2.23	1.90				
	2.38 2.30	2.02 1.96	0.34	14.8	85.2	1.22
54154 F	2.37	1.74				
	2.39 2.38	1.86 1.80	0.58	24.4	75.6	1.14
51589 F	2.42					
	2.49	2.06				
	2.63 2.51	2.13 2.09	0.42	16.7	83.3	1.12
51493 F	2.50					
	2.51	2.29				
	2.56 2.52	2.37 2.33	0.19	7.5	92.5	1.30
55045 F	2.67	2.18				
	2.80 2.74	2.35 2.27	0.47	18.9	81.1	1.16
55026 F	2.75	2.46				
	2.84	2.48				
	2.89 2.83	2.61 2.52	0.31	11.0	89.0	1.14
26054 E	3.80	3.52				
	3.88 3.84	3.58 3.55	0.29	7.6	92.4	1.37
Average				15.6	84.3	1.22
<i>Black Raspberry</i>						
8286 F	2.77	2.10				
	3.08 2.92	2.16 2.13	0.79	27.1	72.9	1.39
33945 F	5.09					
	5.20	4.72				
	5.38 5.26	4.93				
	5.39	4.99 4.88	0.38	7.2	92.8	1.62
<i>Strawberry</i>						
62775 F	1.14	0.74				
	1.15 1.14	0.76 0.75	0.39	34.2	65.8	0.53

TABLE 2.—*Water-insoluble solids and seed content of some authentic berry fruits*

SAMPLE NO.	WATER-INSOLUBLE SOLIDS	SEEDS	WATER-INSOLUBLE SOLIDS—NOT SEEDS	TOTAL NON-SEED	WATER-INSOLUBLE SOLIDS DUE TO SEEDS	AVG WT. 1 SEED	NO. SEEDS 100 g FRUIT	SOLUBLE SOLIDS REFRACTOMETER
	per cent	per cent	per cent	per cent	per cent	Mg		per cent
Subs								
A—Pacific Blackberry	5.00 5.38	3.64 3.69	1.53 3.66	29.5	70.5	1.73	2119	13.2
B—Black Raspberry	9.60 9.64	8.05 8.24	1.47 8.15	15.3	84.7	1.31	6221	16.3
C—New Washington Red Raspberry	5.00 5.26 5.48	3.97 4.09	1.22 4.03	23.2	76.8	1.12	3598	14.5
D—Youngberry	4.59 4.84	3.46 3.66	1.15 3.56	24.4	75.6	3.08	1156	11.7
E—Cuthbert Red Raspberry	6.09 6.14	4.64 4.78	1.40 4.71	22.9	77.1	1.02	4618	13.1
F—Boysenberry	4.09 4.45	3.13 3.32	1.05 3.22	24.6	75.4	3.35	961	12.0
G—Loganberry	6.35 6.57	4.61 4.71	1.80 4.66	27.9	72.1	1.73	2694	13.3

Report average weight of one seed in milligrams, number of seeds per 100 g of sample and after determination of the water-insoluble solids content of the sample, calculate and report the per cent of the total that is due to bare seeds and the per cent that is due to non-seed water-insoluble solids.

The procedure for seeds is more rapid than that for water-insoluble solids. A 50-gram weight of sample can be analyzed for seeds more readily than a 25-gram weight of the same sample can be analyzed for water insoluble solids since difficulties of filtration and washing are eliminated. It may develop that data which include the percentage of seeds in the sample, average weight of the seed, and number of seeds per 100 grams of sample will be more informative and useful in the interpretation of the analyses of berry fruit products than the mere determination of their water-insoluble solids content. Such a conclusion must await the accumulation of authentic data on which comparisons can be made.

Table 1 contains results obtained from the analysis of a number of samples of commercial preserves, including blackberry, loganberry, red raspberry, black raspberry, and strawberry. No conclusions are to be drawn here in regard to the exact proportions of fruit and sugar employed in the preparation of these samples. Some idea can be obtained as to the agreement between duplicates, the relative amounts of seeds to total water-insoluble solids, and the unit weight of seeds.

Table 2 contains a limited amount of information on the water-insoluble solids and seed content of some authentic berry fruits which were analyzed by these procedures.

No report was given on titration of acids, or on fruit acids.

REPORT ON SUGARS AND SUGAR PRODUCTS

By CARL F. SNYDER (National Bureau of Standards, Washington 25, D. C.), *Referee*

RECOMMENDATIONS*

It is recommended—

(1) That the method of the Java Sugar Experiment Station, adopted as a tentative method last year, be made official, with the following changes: "Reagents (f)" should read "dilute sulfuric acid. Three volumes of water plus 1 volume of conc. H_2SO_4 ." Under "Fermentation" the sentence beginning at the end of the fifth line should read: "Place the flask in a water bath kept at $30^\circ C$. and allow to ferment for 4 hours or more, shaking the flask from time to time; if desired, an incubator may be used and the flask may be left overnight."

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 63 (1949).

(2) That the study of methods for the determination of moisture be continued.

(3) That the study be continued on tables of density of solutions of sugar at various temperatures.

(4) That the official method for the determination of free acid in honey, 34.99, be further studied with a view to establishing the end point more accurately.

(5) That the study of methods for the detection of adulteration of honey be continued.

(6) That the method for determination of shellac on confectionery, *This Journal*, 31, 196 (1948), modified as described in this year's report of the Associate Referee, be adopted as official, first action, and that study be continued of methods applicable to confectionery.

(7) That study be continued on the determination of dextrose, maltose, and dextrans, by copper reduction methods in pure sugar mixtures.

(8) That tentative methods, 34.133-34.155, inclusive, be subjected to further study.

(9) That the procedures described in NBS Circular C440, pp. 324-334, for measurement of transmittancy of solutions of commercial sugar products be subjected to collaborative study with a view to their future adoption.

(10) That micro methods for reducing sugars be studied.

(11) That method 34.8 be amended by the following addition: "In liquid products containing invert sugar, correct the per cent solids obtained from 44.7 by adding 0.022 for each per cent of invert sugar present in the sample."

REPORT ON UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

By F. W. ZERBAN (New York Sugar Trade Laboratory, Inc., New York, N. Y.), *Associate Referee*

At the 1947 meeting of the Association the Java method was adopted as a tentative method for the determination of unfermented reducing substances in molasses.¹ In accordance with the recommendation made, the collaborative work has been repeated this year, by the method as published.² Samples of a raw sugar blackstrap and a refiners' blackstrap were sent to nine collaborators who had expressed willingness to carry out the analyses. Reports have been received from all of these. The results are shown in Table 1.

The deviations of the individual averages from the grand averages are given in Table 2.

¹ *This Journal*, 31, 61 (1948).

² *This Journal*, 31, 192 (1948).

TABLE 1.—*Percent unfermented reducing substances,
expressed as invert sugar
In raw sugar blackstrap and refiners' blackstrap*

ANALYST	RAW SUGAR BLACKSTRAP	REFINERS' BLACKSTRAP
1. Sam Byall, New Orleans, La.	4.42 4.31 4.25 4.42	3.75 3.64 3.87 3.70
2. J. K. Dale, Terre Haute, Ind.	4.20 4.25 4.09 4.14	3.64 3.64 3.64 3.61
3. Carl Erb (New York yeast), New York, N. Y.	4.40 4.46	3.82 3.82
4. Same, (New Orleans yeast)	4.32 4.26	3.71 3.71
5. W. J. Hughes, New York, N. Y.	4.35 4.40	3.74 3.80
6. W. L. Porter, Philadelphia, Pa.	4.30 4.35 4.32	3.80 3.81 3.92
7. F. E. Randall, Buffalo, N. Y.	4.64 4.64 4.70 4.76	4.20 4.20 4.25 4.14
8. D. J. Smith, Boston, Mass.	4.24 4.28 4.31	3.75 3.80 3.81
9. W. O. Winkler, Washington, D. C.	4.48 4.48	3.92 3.93
10. R. T. Wisthoff, Baltimore, Md.	4.47 4.47 4.42 4.36 4.42 4.36	3.77 3.77 3.71 3.82 3.87 3.82
Averages		
1	4.35	3.74
2	4.17	3.63
3	4.43	3.82
4	4.29	3.71
5	4.38	3.77
6	4.32	3.84
7	4.68	4.20
8	4.28	3.79
9	4.48	3.93
10	4.42	3.79
Grand Averages	4.38	3.82
Grand Averages, omitting No. 7	4.35	3.78

TABLE 2.—*Deviations of individual averages from grand averages*
Per cent of invert sugar

ANALYST	RAW SUGAR BLACKSTRAP	REFINERS' BLACKSTRAP	NUMERICAL AVERAGE
1	-0.03	-0.08	0.055
2	-0.21	-0.19	0.200
3	+0.05	0.00	0.025
4	-0.09	-0.11	0.100
5	0.00	-0.05	0.025
6	-0.06	+0.02	0.040
7	+0.30	+0.38	0.340
8	-0.10	-0.03	0.065
9	+0.10	+0.11	0.105
10	+0.04	-0.03	0.035
Average deviations	+0.049	+0.051	
	-0.049	-0.049	
	±0.098	±0.100	

The maximum spread between the results of repeated determinations by any of the individual analysts is 0.17 for the raw sugar blackstrap, and 0.23 for the refiners' blackstrap; the maximum differences for the ten sets of analyses average 0.085 and 0.081, respectively.

The results of the collaborators check satisfactorily, except for Analyst No. 7, who had no previous experience with the method. If his results are omitted and the other nine are averaged, the maximum deviation from the grand averages is only 0.18 for the raw sugar blackstrap, and 0.15 for the refiners' blackstrap. The average deviation is only ± 0.065 and ± 0.054 , respectively. This is very good agreement for analyses of this nature, requiring many manipulations.

The results also show that the particular lot of Fleischmann's baker's yeast, produced in different localities, has no noticeable effect. Sam Byall (New Orleans), who in previous years had obtained unusually high results, and D. J. Smith (Boston), whose results had been unusually low, checked well this time with each other and with the averages, on both samples. Carl Erb (New York) ran analyses with yeast secured in New York, and also with yeast sent by Mr. Byall in a well insulated package via air mail from New Orleans. The results were in good agreement with the averages.

W. L. Porter and R. T. Wisthoff have called attention to the indistinctness of the starch end point in the iodine titration, and J. K. Dale had mentioned this point in earlier work. This difficulty, according to Mr. Wisthoff, can be overcome by acidifying with sulfuric acid, diluted with 3 instead of 5 volumes of water. This observation has been confirmed in this laboratory.

W. L. Porter has reported that four hours was insufficient for complete fermentation. The directions adopted last year read: "for at least 4 hours, shaking the flask from time to time. When fermentation is complete, etc." To avoid misunderstanding, this should be changed to read as stated below.

RECOMMENDATIONS*

It is recommended that the method of the Java Sugar Experiment Station, adopted as a tentative method last year, be made official, first action, with the following changes:

REAGENTS, (f) should read: "*Dilute sulfuric acid.*—3 volumes of water plus 1 volume of conc. H_2SO_4 ."

Under FERMENTATION, the sentence beginning at the end of the fifth line should read: "Place the flask in a water bath kept at 30°C. and allow to ferment for 4 hours or more, shaking the flask from time to time; if desired, an incubator may be used, and the flask may be left overnight. When fermentation is complete, etc."

REPORT ON CONFECTIONERY

By CHARLES A. WOOD (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

According to the recommendation of last year,¹ some further investigation was made of the lac method with reference to the recovery of relatively large amounts of added shellac. On checking the solubility of the lac in the isoamyl alcohol-benzol mixture, at times on cooling and standing during the water wash, some of the lac settled out. To obviate this difficulty, the method² was changed by inserting the words "hot (about 60°)" before "water" in line 18 of the method, changing "reject the wash water" to "filter wash water if necessary" in line 19, and changing "filter" to "filters" in line 22. In line 15 following isoamyl alcohol, "B.P. 129-132" was inserted.

Three samples were prepared for collaborative testing using the same shellac and sugar candy as employed last year.

Sample A—Sugar candy with 0.90% lac added.

Sample B— " " " 0.67% " "

Sample C— " " " 0.45% " "

Results are shown in Table 1.

The results on the higher lac samples now seem fairly good. There will always be some tendency to low results, since drying the shellac for an

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 64 (1949).

¹ *This Journal*, 31, 61, 1948.

² *This Journal*, 31, 196, 1948.

TABLE 1.—*Collaborative results*

COLLABORATOR	LAC FOUND		
	A	B	C
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
J. L. Hogan	0.89	0.58	0.42
	0.90	0.60	0.44
C. A. Wood	0.90	0.66	0.44
	0.90	0.65	0.44
Average	0.90	0.62	0.435

appreciable time gradually changes it to a benzol alcohol insoluble form.

It is recommended*—

(1) That the method as published in *This Journal*, 31, 196 (1948) with modifications as indicated above be adopted as tentative.

(2) That further collaborative work be done.

REPORT ON REDUCING SUGARS

By EMMA J. McDONALD (National Bureau of Standards, Washington 25, D. C.), *Associate Referee*

A collaborative study has been made of Ofner's Method for the determination of small quantities of invert sugar in the presence of sucrose. This method is now a tentative method of the Association of Official Agricultural Chemists.

Four analysts were provided with three different sucrose samples and with levulose and dextrose. Analyses were made in duplicate on the three sucrose samples and determinations were also made on 10, 5, and 1 mg. of invert sugar. The duplicate results obtained by the different collaborators were in good agreement, differing by approximately 0.1 mg. Bearing in mind this consistency within the results of each analyst, the overall results of the collaboration can be considered. The pure invert analyses agreed with the invert present within an average of 0.2 mg. with a maximum error of 0.5 mg. Sucrose samples I and II contained an average of 4.7 and 2.2 mg. of invert in 10 grams of sugar, or .047 and .022 per cent of invert, respectively. The results of the various analysts differed by .005 per cent and .003 per cent invert, respectively, in the two samples. Sucrose sample III was reported to contain 0.1 and 0.2 mg. of invert per 10 g. of

* For report of Subcommittee D and action of the Association see *This Journal*, 32, 63 (1949).

sugar by two investigators and 0.8 and 1.0 mg. by the remaining two. There is some question as to the stability of the sample since the first two analysts obtained their results 5 months prior to those reporting the higher percentages of invert. A summary of the collaborative work is given in the following table.

TABLE 1.—*Collaborative results*

	SUCROSE I	SUCROSE II	SUCROSE III	10 MG INVERT	5 MG INVERT	1 MG INVERT
Analyst A	4.85 4.85	1.98 2.04	0.11 0.18	9.96 9.88	5.06 5.12	0.91 0.88
Analyst B	3.70 3.74	2.39 2.12	0.23 0.18	10.07 10.07	5.04 5.14	0.97 1.03
Analyst C	5.66 5.49	2.65 2.77	0.93 1.03	10.20 10.34	5.52 5.14	1.42 1.39
Analyst D	4.76 4.80	1.85 1.89	.84 .84	9.63 9.60	4.99 4.94	.96 .98
	SUCROSE I			SUCROSE II		SUCROSE III
Average Invert	4.73 mg			2.21 mg		.42 mg
Average Deviation	± .51 mg			± .29 mg		± .32 mg
	Invert					
Average Error	10 mg			5 mg		1 mg
	± .20			± .14		± .14

Ofner's method uses a carbonate copper solution. The reduced copper is not filtered but is determined in the reaction mixture by the addition of iodine and back titration with thiosulfate. The mild action of the carbonate solution compared with that of alkali reagents on sucrose, the fact that the reduced copper can be determined in the reaction mixture, and finally, and of greatest importance, the consistency of results obtained by different analysts are the basis on which it is recommended* that Ofner's method be made an official method.

No reports were given on drying methods, densimetric and refractive methods, honey (free acid and commercial syrup adulterants), corn syrup and corn sugar, color and turbidity in sugar products, or micro sugar methods.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 64 (1949).

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

No collaborative work has been reported on any of the several projects under this refereeship for this meeting. However, plans have been drawn up which should result in definite progress during the coming year.

Mr. R. D. Stanley, Associate Referee on Organic Solvents in Flavors, has made an informal report to the Referee concerning a considerable amount of work on procedures designed to remove acetone from products containing both acetone and isopropyl alcohol, to make it possible to apply the method given in *Methods of Analysis*, 25.26, which is applicable only when acetone is absent. So far the work is encouraging but not conclusive and a definite answer to the problem is expected before the next meeting.

No report has as yet been received on Maple Flavor Concentrates.

The Referee is recommending the continuation of all previously recommended projects with the addition of a new item on collaborative study of the tentative procedures for vanilla resins.

RECOMMENDATIONS*

It is recommended—

(1) That the collaborative study of the reflux method for determination of peel oil in citrus fruit juices and the use of the modified oil separation trap be continued.

(2) That collaborative work be continued on the method for determination of beta-ionone where small amounts are present.

(3) That collaborative studies on the Ripper method for determination of aldehydes in spirits as applied to lemon oils and extracts be continued.

(4) That collaborative studies of the methods proposed by the Referee for determination of esters in lemon extract be continued.

(5) That collaborative studies on the Seeker-Kirby Method for determination of esters in lemon and orange oils (Dept. of Agri. Bull. 241) be continued.

(6) That collaborative studies of methods for extract containing both isopropyl alcohol and acetone be continued.

(7) That collaborative study of the photometric method for determination of vanillin and coumarin be continued.

(8) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts, with special reference to the automatic extraction of vanillin and coumarin.

(9) That the study of emulsion flavors be continued.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 62 (1949).

(10) That studies on maple concentrates and imitations be continued.

(11) That study of the method for determination of diacetyl, published in *This Journal*, 25, 255, be continued.

(12) That the Referee study collaboratively the modification of 25.23 as given in last year's report (*This Journal*, 31, 202 (1948)).

(13) That the Referee study collaboratively the modification of 25.54 as given in last year's report (*This Journal*, 31, 203 (1948)).

(14) That the methods for vanilla resins in vanilla extract, 25.15 and 25.16, be studied collaboratively.

No report was given on beta-ionone, lemon oils and extracts, organic solvents, vanillin, emulsion flavors, maple flavor concentrates, or diacetyl.

REPORT ON FERTILIZERS

By F. W. QUACKENBUSH (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Referee*

During the year, one Associate Referee was appointed to study methods of analysis for inert materials in fertilizers. Most of the Associate Referees previously appointed have done some work on problems related to their respective assignments. However, not all of them have reported.

Considerable interest has been shown during the year on problems of sampling and segregation of fertilizer materials. Research has been initiated at the Indiana and Maryland laboratories to determine the correct number of cores to be drawn from a given lot of fertilizer in order that a statistically sound sample can be obtained. It is hoped that the results of these studies will stimulate other States to carry out similar experiments, and that by the end of another year we will have established without question the correct number of bags which an inspector should sample under a given set of circumstances.

RECOMMENDATIONS*

Sampling.—The recommendations of the Associate Referee, which are as follows, are approved:

(1) That study of sampling equipment and method of sampling be continued.

(2) That preparation of sample for analysis be studied.

Phosphoric Acid.—The recommendations of the Associate Referee are approved.

Moisture.—The recommendations of the Associate Referee are approved.

Nitrogen.—The recommendations of the Associate Referee, which are as follows, are approved:

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 43 (1949).

(1) That the formaldehyde titration method be adopted as official, first action, for determining nitrogen in ammonium nitrate.

(2) That the study of nitrogen in high nitrate-chloride mixtures be continued.

Potash and Platinum Recovery Methods.—The recommendations of the Associate Referee, which are as follows, are approved:

(1) That a survey be made of the different types of mills being used for preparation of the sample.

(2) That collaborative potash work on samples prepared by the different mills be conducted on a greater variety of samples.

No reports were received on seven subjects:

Magnesium and manganese
Acid- and base-forming quality
Potash and platinum recovery methods
Sulfur
Copper and zinc
Boron
Inert materials

It is recommended* that work of all Associate Referees be continued.

REPORT ON SAMPLING FERTILIZERS

By H. R. ALLEN (Kentucky Agricultural Experiment Station, Lexington, Kentucky), *Associate Referee*†

This report consists of results of (1) a questionnaire on sampling fertilizers and preparation of samples for analysis; (2) a collaborative study of the effectiveness of 3 kinds of sample containers in preventing change in moisture; (3) a comparison of the slotted single-tube and the slotted double-tube sampler in taking samples; (4) a comparison of preparation of samples for analysis with and without screening the sample through 10-mesh.

QUESTIONNAIRE ON SAMPLING FERTILIZERS

It was felt that work on this subject would be aided by a knowledge of the present methods used in all States of collecting samples and preparing them for analysis. This questionnaire was sent to the fertilizer control officials in each State and in the Dominion of Canada. Replies with questionnaire filled out were received from 41 officials. Five State officials replied but did not fill out the questionnaire, chiefly because only a few samples were taken. Answers were not complete in a few instances.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 43 (1949).

† This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.

Results of the questionnaire are given in Table 1. They show considerable variation in procedures used in the different States. One of the greatest variations is in the number of bags sampled. A number of States sample a smaller number of bags than is prescribed by the A.O.A.C. procedure.

TABLE 1.—*Questionnaire on sampling fertilizers*
(Numbers in column at right refer to number of States)

I. METHOD OF COLLECTING SAMPLES

1. Type of sampler used:	
A. Use single-tube sampler	20
B. Use double-tube sampler	17
C. Use tube-and-rod sampler	4
2. Number of bags sampled:	
A. Less than 10 bags present:	
All bags sampled	30
6 bags sampled	5
3 or 4 bags sampled	1
1 bag sampled	2
No bags sampled	2
B. From 10 to 100 bags present:	
10 bags sampled	27
10 per cent of bags sampled	3
5 bags sampled	3
4 to 20 bags sampled	2
20 per cent sampled	1
15 bags sampled	1
10 to 20 bags sampled	1
C. More than 100 bags present:	
10 per cent of bags sampled	15
A.O.A.C. procedure* followed	10
10 bags sampled	6
20 bags sampled	3
5 bags sampled	2
20 to 40 bags sampled	1
5 bags plus 1 bag for each ton over 5	1
3. Method used in taking sample:	
A. 1 core from each bag parallel to sides	25
B. 2 or more cores from each bag parallel to sides	4
C. Cores taken diagonally	17
4. Samples sent to laboratory:	
A. Entire sample	17
B. A portion of sample	24
5. Weight of sample sent to laboratory:	
A. Less than 1 pound	4
B. 1 to 2 pounds	27
C. 2 pounds or more	10

* Before changed at last meeting.

6. Container for samples:

A. Type of container in which samples are sent to laboratory:

Glass containers	21
Paper containers†	11
Paste-board or ice-cream containers	7
Cotton bag (paraffined)	2
Metal cans (2 States use more than one type)	2

B. Type of container in which reserve portion is stored:

Glass containers	27
Waxed ice-cream containers	3
Metal can	1
Paper bag with liner	1
Do not save reserve	2
No answer	4

7. Are liquid fertilizers sampled?

Yes	26
No	11

A. Type of sampler used:

Sample small packages and purchase 1 package	18
Sample large containers, using one of following: steel bomb, rubber tube, stainless steel tube, syphon, modified milk sampler, stainless steel dipper	8

II. METHOD OF PREPARING SAMPLES FOR ANALYSIS

1. Mesh of screen through which inspector's sample is passed before it is subdivided for laboratory sample:

A. 2 mm. or 10 mesh	13
B. 20 mesh	2
C. Samples not screened	17

Note—Whole sample ground in 2 States.

2. Weight of sample prepared for laboratory:

A. Less than $\frac{1}{4}$ pound	10
B. $\frac{1}{4}$ to $\frac{1}{2}$ pound	20
C. $\frac{1}{2}$ to 1 pound	8

3. Size of screen through which ground sample is passed:

A. 1 mm. circular opening	14
B. 20 mesh	7
C. 0.5 mm. circular opening or finer than 20-mesh	13

4. Method of grinding samples:

A. Mortar and pestle used	15
B. Mill used	23

NOTE 1. 8 using mill also use mortar and pestle when necessary.

NOTE 2. 6 use mortar and pestle who would use mill if a satisfactory one is developed.

NOTE 3. 10 Mikro-samplmills are in use.

† Some States use inner liner.

A number of States send the whole sample collected by the inspector to the laboratory. When a large stock of fertilizer is sampled, 30 or more sample cores are required and a larger container is needed if the whole sample is sent in. It seems essential to develop a suitable container for such samples. The container should be one which would keep the moisture content of the sample unchanged. A container which is easily transported to place of sampling is desirable. A number of States now use ordinary paper bags, some of which have an inner liner.

The questionnaire results show that about 8 States sample liquid fertilizers in larger than package quantity, using a variety of sampler types. A number of States do not screen the inspector's sample through 10-mesh as prescribed by the A.O.A.C., before grinding the portion for the laboratory. Three or more States grind the whole sample, in which case the above provision is unnecessary.

While a number of States still use mortar and pestle for grinding samples the use of mills seems to be increasing. A number now using mortar and pestle stated that they would use a mill when a satisfactory one is developed. Ten or more States now use the new "Mikro-samplmill."

Comments of Officials.—Twenty officials were satisfied with the present procedures for taking samples and preparing same for analysis, and nine were not satisfied. Many offered suggestions for study. Among them, six thought study should be made to determine the least number of bags necessary to give a representative sample, three listed need of suitable container in which to ship sample to the laboratory, three listed sampling and preparation of sample in general, one thought a suitable sample divider for use in the field would be desirable, and one suggested a study of mixing and quartering sample in the field versus sending in the whole sample.

COLLABORATIVE STUDY OF SAMPLE CONTAINERS

Through the assistance of Dr. S. F. Thornton, the Bemis Brothers Bag Company developed a double-wall asphalt-impregnated sample bag made of heavy paper. This bag measures 4 by 6 inches on the bottom and it is about 12½ inches tall. It will hold 30 to 35 average sample cores.

Collaborative work was conducted on moisture change in samples stored in a quart fruit jar, in the Bemis bag, and in an ordinary nail sack in a moist and in a dry atmosphere, under partially controlled humidity conditions.

The collaborators were:

- (1) Leo Fanuef and Kenneth Helrich, Agricultural Experiment Station, Rutgers, N. J.
- (2) J. W. MacKay, North American Cyanamid, Ltd., Niagara Falls, Canada.
- (3) Philip McG. Shuey, Shuey & Co., Savannah, Ga.
- (4) C. T. McCloud, F. S. Royster Guano Co., Norfolk, Va.
- (5) H. R. Allen, Agricultural Experiment Station, Lexington, Ky.
- (6) H. K. White, Purdue Agricultural Experiment Station, Lafayette, Indiana.

DIRECTIONS FOR COLLABORATIVE STUDY

Procure at least 3 quarts of a mixed fertilizer, preferably one containing 5 or 6 per cent nitrogen. The mixture may be sampled in the regular way or it may be composed of a composite of your reserve samples. Grind whole amount (not too fine, preferably to pass a 1 mm sieve). Mix well, spread out, and divide whole amount into 3 portions. Take a representative sample for moisture determination from each portion and place each in a separate airtight sample bottle. It is best to mix each portion again before taking this sample.

Place 1 portion (about 1 quart) in a Mason jar with zinc top and rubber gasket, and screw lid tight. Place equal portions in the Bemis bag (No. 2) and in the nail sack (No. 3). Fold the top of each bag twice and use paper clips (5 or 6) to keep tight. The side edges may be sealed with adhesive tape.

Place the 3 containers under a bell jar on a glass plate bottom. Put a 250 ml beaker of water at room temp. and a relative-humidity indicator and thermometer under the bell jar. Let sample stay under the bell jar for 4 days. The humidity indicator can be dispensed with if not available. If used, take humidity and temperature readings at beginning and end of each test.

Take out samples, mix quickly on oilcloth, and take a portion of each for moisture determination as before. Put samples back into their respective containers, seal as before, and place in a warm, dry atmosphere for 3 days (about 2 feet from the window getting direct sunlight for part of the day is satisfactory). A source of heat such as a small hot-plate may be used near the samples for short periods to dry the air if it is too moist. Place the humidity-indicator near the samples and take readings. If samples are warm at end of test, remove to a cooler place for an hour, then mix and take portions for moisture determinations as before.

Make all moisture determinations for each group as soon as possible after placing portions in sample bottle. Use a 2-gram sample and place it in an air oven for 3 hours at 98° to 100°C. Use dishes equipped with covers while cooling in desiccator, preferably the aluminum dish (Fisher-E. & A. catalog No. 8-722). Report as per cent moisture.

Please report any necessary variations from these directions. If bell jar is not available, use some substitute, such as a cardboard box.

If time permits, report moisture tests on a sample of commercial ammonium nitrate, except do not grind whole portion. Grind small amounts quickly for moisture determinations.

Results of collaborative study are given in Table 2. Since each collaborator used his own samples, the results of each must be studied as a unit. Some of the divergent results are probably due to changes in moisture during mixing to obtain portion for moisture determination. To reduce such change, it was specified that all samples be ground previous to the tests. Collaborators 2, 5, and 6 reported very little change in moisture of the samples stored in glass or in the Bemis bag, either in the moist or dry atmosphere. The same collaborators found that the sample stored in the nail sack gained from 0.68 to 4.50 per cent moisture in the moist atmosphere and lost 1.25 to 4.88 per cent moisture in the dry atmosphere.

Collaborators 1 and 3 found the sample in the nail sack lost appreciable moisture in the dry atmosphere but did not gain it in the moist atmosphere. Collaborator 4 found the sample in the nail sack gained 2.25 per cent moisture in the moist atmosphere but that the moisture remained the same in the dry atmosphere.

TABLE 2.—*Collaborators results in percent moisture on moisture tests with different containers*

COLLABORATOR NUMBER	CONTAINER	BEFORE TEST	MOIST ATMOSPHERE	CHANGE	DRY ATMOSPHERE	CHANGE
1	<i>Sample 1, 7-7-7 Grade</i>					
	Quart jar	5.72	5.62	-0.10	5.56	-0.06
	Bemis bag	5.65	5.64	-0.01	5.45	-0.19
	Nail sack	5.38	5.39	+0.01	5.07	-0.32
	<i>Sample 2, Same</i>					
	Quart jar	5.55	5.76	+0.21	5.54	-0.22
	Bemis bag	5.64	5.69	+0.05	5.54	-0.15
	Nail sack	5.52	5.43	-0.09	4.76	-0.67
2	<i>Sample 1, Mixed Fertilizer</i>					
	Quart jar	4.98	4.98	0.00	4.97	-0.01
	Bemis bag	4.98	4.99	+0.01	4.97	-0.02
	Nail sack	4.99	5.67	+0.68	2.64	-3.13
	<i>Sample 2, Aeroprills</i>					
	Quart jar	0.20	0.20	0.00	0.19	-0.01
	Bemis bag	0.20	0.22	+0.02	0.21	-0.01
	Nail sack	0.19	1.49	+1.30	0.24	-1.25
3	<i>Sample 1, Mixed Fertilizer</i>					
	Quart jar	7.94	7.50	-0.44	7.27	+0.23
	Bemis bag	7.94	7.61	-0.33	7.59	-0.02
	Nail sack	7.94	7.97	+0.03	6.89	-1.08
4	<i>Sample 1, 5-10-5 Grade</i>					
	Quart jar	5.95	5.90	-0.05	5.85	-0.05
	Bemis bag	5.90	6.20	+0.30	6.15	-0.05
	Nail sack	5.85	8.10	+2.25	8.05	-0.05
5	<i>Sample 1, 6-8-6 Grade</i>					
	Quart jar	3.75	3.67	-0.08	3.55	-0.12
	Bemis bag	3.75	3.78	+0.03	3.52	-0.26
	Nail sack	3.82	4.80	+0.98	2.54	-2.26
COLLABORATOR NUMBER*	CONTAINER	SAMPLE NUMBER	MOIST ATMOSPHERE PER CENT CHANGE	DRY ATMOSPHERE PER CENT CHANGE		
6	Quart jar	1	0.00	0.00		
	Quart jar	2	0.00	0.00		
	Quart jar	3	0.00	0.00		
	Bemis bag	1	+0.37	-0.21		
	Bemis bag	2	0.00	-0.16		
	Bemis bag	3	+0.29	-0.48		
	Nail sack	1	+4.50	-3.86		
	Nail sack	2	+4.07	-4.88		
	Nail sack	3	+1.60	-4.70		

* Collaborator 6 weighed the whole sample and container in each case and compared per cent change from the gain or loss in weight. Sample 1 was 8-8-8 grade, Sample 2 was 4-12-4, and Sample 3 was 5-10-5.

Collaborator 6 could not obtain uniform results by following the directions. He attributed this to loss or gain while taking sample for moisture determination. He modified the procedure as follows: Samples were placed in the containers as directed, and the whole sample and container weighed in each instance. Then containers and samples were placed in desiccators, connected to a pump forcing air of 100 per cent relative humidity through the desiccators in the moist atmosphere test, and air of 0 to 8 per cent humidity in the dry atmosphere test. Gain or loss of weight of each container plus sample was recorded. Reported weights were corrected for gain or loss of moisture due to the containers.

Humidity readings were taken by only 2 collaborators, so they have been omitted. The results show that the Bemis bag is almost as effective in moisture resistance as the glass fruit jar sealed with the rubber gasket, and that it is greatly superior to the nail sack for this purpose. It is believed that folding the top of the bag twice and use of ordinary letter clips or a stapling machine makes a satisfactory closure.

COMPARISON OF SINGLE-TUBE AND DOUBLE-TUBE SAMPLERS

Previous study¹ showed little difference in samples obtained with these two types of samplers when the fertilizers were ordinary mixed goods. It was suggested that the samplers be compared under more extreme conditions.

A wood box, open at the top, was built. The inside dimensions were: 23 inches long, 5 inches wide, and 5 inches deep. Two holes side by side were bored in each end and in a partition placed $1\frac{1}{2}$ inches from the front end. The holes were lined up so the inserted samplers were parallel to sides of the box. Holes in the inner partition served as a guide for the samplers. Paper was glued on the holes before placing sample in the box to prevent loss of sample. In sampling, samplers were pushed through the rear holes so that only the slot of each sampler was within the box.

Materials used were ammonium sulfate, superphosphate, and muriate of potash. Materials were placed in the box in 8 blocks of 895 grams each. Four of the blocks were superphosphate, 2 were ammonium sulfate, and 2 were muriate of potash. The order of the blocks from front to rear were superphosphate, ammonium sulfate, superphosphate, muriate of potash, ammonium sulfate, superphosphate, muriate of potash, superphosphate.

The 2 samplers were the ones described in a previous paper.¹ The samplers were inserted at the same time by 2 operators, the single-tube sampler with the slot down and the double-tube sampler with the slot closed. The single-tube sampler was turned over, the double-tube sampler was opened and the sample was taken. The slot was closed and both samples were removed. Cores of samples from this sampling were placed

¹ Allen, H. R., *This Journal*, 31, 205-209 (1948).

in sample bottles and the operation repeated to obtain a second and third set of cores.

The sample materials in the same proportion were thoroughly mixed, the mixture was placed in the box and 3 sets of cores taken as before. Samples were ground and analyzed for nitrogen, total phosphoric acid, and potash. Results are given in Table 3.

TABLE 3.—Comparison of results on samples obtained with slotted single-tube and slotted double-tube samplers. Materials sampled in blocks (unmixed) and in mixed form

Results in percent

TYPE OF SAMPLE	SAMPLER	CORE NUMBER	NITROGEN	TOTAL PHOS- PHORIC ACID	POTASH
Block*	single-tube	1	2.09	10.50	24.58
Block	double-tube	1	2.40	10.65	22.94
Block	single-tube	2	1.86	11.90	20.78
Block	double-tube	2	1.55	11.50	22.78
Block	single-tube	3	3.96	12.45	14.86
Block	double-tube	3	3.63	11.10	18.22
Mixture	single-tube	1	5.40	9.75	16.88
Mixture	double-tube	1	5.38	9.90	16.86
Mixture	single-tube	2	5.36	9.95	16.90
Mixture	double-tube	2	5.39	9.90	16.90
Mixture	single-tube	3	5.43	9.80	16.92
Mixture	double-tube	3	5.39	9.80	16.88

* Blocks were composed of 895 g each in this order from front to rear: superphosphate, ammonium sulfate, superphosphate, muriate of potash, ammonium sulfate, superphosphate, muriate of potash, superphosphate.

Results on materials in blocks would not be the theoretical percentages because the same weights were used in each block and, since the materials differed in density, they did not occupy equal spaces. Equal volumes of the materials in the order ammonium sulfate, superphosphate, and muriate of potash were in the ratio 4.5:5.3:7.5. This does not seem sufficient to cause the large difference in results obtained from this sampling. Results for the mixed samples were markedly uniform. The experiment did not indicate that either sampler was more accurate than the other. It did indicate in uniform mixtures there is practically no difference in analyses of samples taken with the 2 types of samplers and that insertion of the samplers several times in the same space does not change concentration of nutrients in that space. It is believed experiments of this type might be continued to advantage.

COMPARISON OF PREPARATION OF SAMPLES FOR ANALYSIS,
WITH AND WITHOUT SCREENING WHOLE SAMPLE
THROUGH 10-MESH

Replies to the questionnaire show many laboratories do not screen the whole sample through 10-mesh before grinding the portion for analysis. In this laboratory previous practice has been to grind the whole sample (1 quart), in which case screening is not necessary. Recently one of the new Mikro-samplmills was installed and, while it will grind a quart or more of sample, this is not very practical, especially for samples with excessive moisture. The following procedure was tried in order to check accuracy of results obtained when the whole sample was not screened through 10-mesh.

All samples were well mixed and large lumps were broken up with spatula. One-half pint of each sample (one-fourth of whole sample) was ground in the Mikro-samplmill and usual analyses made. On each sample showing a deficiency of 0.50 per cent in any guarantee, all the reserve portion was screened through 10-mesh, and the coarse portion was ground as before. Comparison of results from the two procedures is given in Table 4.

TABLE 4.—*Comparison of analyses of samples with and without screening through 10-mesh**

DIFFERENCE IN PER CENT IN CHECK ANALYSES	NUMBER OF CHECK ANALYSES ON—		
	NITROGEN	AVAILABLE PHOSPHORIC ACID	POTASH
0-0.05	12	9	17
0.60-0.10	5	3	5
0.11-0.15	3	4	6
0.16-0.20†	0	6	6
>0.20	0	4	1
Totals	20	26	35

* Original analyses: One-fourth of quart sample ground in Mikro-samplmill without screening.
Check analyses: Remainder of quart sample screened through 10-mesh, coarse part ground in mill,
and one-fourth quart of mixture ground.
† 94 per cent of checks agree within 0.20 per cent.

The results show that 63 per cent of the checks agree within 0.10 and 94 per cent agree within 0.20. In most cases only one analysis was made on each portion of the sample and some of the differences could be due to slight analytical errors. Duplicate analyses on each portion might reduce the above differences.

Sampling is time-consuming and elimination of the screenings process reduces sampling time by one-half or more. It is assumed that even better checks would be obtained if one-half the sample is ground. This laboratory

has found the Mikro-samplmill very satisfactory. A pint sample can be ground in about 1 minute (exclusive of cleaning mill). The mill is easier to clean than any others used. All mixed fertilizers and superphosphates for the 1948 season to date have been ground in this mill.

ACKNOWLEDGMENT

The phosphoric acid analyses shown in Table 3 were made by Lelah Gault and the potash analyses in Table 4 were made by members of the laboratory staff of this Station.

SUMMARY

Results of a questionnaire on sampling and preparation of sample for analysis are tabulated.

Number of bags sampled and preparation of sample for analysis varied most in the different States. Replies indicate much interest in investigation of various parts of the procedures.

Collaborative study on sample containers in which samples are sent to the laboratory shows the experimental Bemis bag is about as effective as glass in keeping moisture of the sample unchanged.

Comparison of the single-tube and double-tube sampler, using a specially built sample box, did not show that either type sampler was more accurate than the other.

Comparison of preparation of samples for analysis with and without screening whole sample through 10-mesh showed that 80 per cent of analyses using the two procedures agree within 0.15 and that 94 per cent agree within 0.20.

Much time is saved when the screening process is omitted.

RECOMMENDATIONS*

It is recommended—

- (1) That study of sampling equipment and method of sampling be continued.
- (2) That preparation of sample for analysis be studied.

* For the report of Subcommittee A and action by the Association, see *This Journal*, 32, 43 (1949).

REPORT ON PHOSPHORIC ACID IN FERTILIZERS:
COMPARISON OF NEUTRAL AMMONIUM CITRATE
AND TWO PER CENT CITRIC ACID SOLUTIONS AS
SOLVENTS FOR BASIC SLAG

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In 1886 Paul Wagner (40) proposed the use of an acid ammonium citrate solution for the determination of available P_2O_5 in superphosphate and precipitated phosphate. For the evaluation of the P_2O_5 content of basic slag he (41) recommended in 1894 the use of a solution containing 60 grams of crystallized citric acid and 11.17 grams of ammonia per liter, the composition of which corresponds to 14 grams of free citric acid per liter. In 1896 Gerlach and Passon (9) reported that in tests on 84 samples of basic slag the same results for soluble P_2O_5 were usually obtained by the use of a solution containing no ammonia and only 14 grams of citric acid as by the use of Wagner's acid ammonium citrate. They further reported that a solution containing 46 grams of citric acid and 11.17 grams of ammonia per liter (corresponding to Wagner's solution without the free citric acid) gave very much lower results than did either Wagner's acid ammonium citrate or a 1.4 per cent solution of free citric acid. Gerlach and Passon concluded that free citric acid and not ammonium citrate is the active solvent for slag P_2O_5 , a conclusion which is not supported by the work of Jacob, Rader, and Tremearne (24) 40 years later. On the basis of a large number of plant-growth tests, Wagner (42) recommended, in 1899, the use of a 2 per cent citric acid solution for the evaluation of basic slag, and a detailed discussion of the method was given in a later publication (43).

Beginning in 1896 with Huston's and Jones' (21) paper on the action of ammonium citrate and citric acid on basic slag, this Association's work on slag prior to 1911 has been summarized by Haskins and Patten (16). On the basis of further extensive laboratory, pot, and field experiments (15, 16, 32, 45), Wagner's 2 per cent citric acid method for the evaluation of this material was officially adopted in 1922 (12, 14, 34). The plant-growth experiments were made under the direction of the Committee on Vegetation Tests on the Availability of Phosphoric Acid in Basic Slag. The concluding paragraph of the final report (15) of the Committee is as follows:

"The results obtained by the experiment have established the fact that all four slags contained their phosphoric acid in forms freely available to the crops grown, comparing favorably, both in yield of crop and in phosphoric acid recovered, with results obtained with acid phosphate. Moreover, the availability figures established by the

vegetation pot work compare favorably with the available phosphoric acid as measured by the Wagner method for Thomas slag phosphate and the official neutral citrate of ammonia method for acid phosphate or superphosphate."

Although the Association's work that finally led to official adoption of the Wagner method was done on Thomas-Bessemer slag the procedure has subsequently been used also for the evaluation of open-hearth and other types of slag.

For some 35 years after the publication of the paper by Gerlach and Passon (9) it seems to have been the general opinion that, as compared with 2 per cent citric acid, ammonium citrate solutions are not satisfactory solvents for the P_2O_5 of basic slags (11, 39, 44). More recently, however, it has been shown that the solubility of the P_2O_5 in neutral ammonium citrate solution depends to a marked extent on the ratio of sample weight to solvent volume (23, 24, 37). Thus, the citric acid solubility is usually much higher than the neutral citrate solubility when the latter is determined on the basis of 2 grams of sample per 100 ml. of solvent in accordance with the former official method for citrate-insoluble P_2O_5 (*Methods of Analysis*, 1930, pp. 17-18), whereas there is evidence (17, 18, 23, 24, 25, 36, 37) that the difference is small when the weight of sample per 100 ml. of citrate solution is reduced to 1 gram, as directed in the present procedure (*Methods of Analysis*, 1945, pp. 23-24).

In view of the indicated close agreement between the solubility values obtained by the 2 per cent citric acid method and the present official citrate procedure the Referee on Fertilizers in his report to the 1946 meeting of the Association (8) recommended "that a study be made of the applicability of the ammonium citrate method to basic slag, with the object of adopting it in place of the citric acid method if such change is found to be desirable." Pursuant to this recommendation a collaborative investigation of the subject was carried out and the results are presented herein.

SAMPLES

The samples submitted to the collaborators are listed in Table 1, which also shows the total P_2O_5 and the fluorine content of the materials. Table 2 gives the mechanical composition of 11 of the 14 samples as received from the manufacturers and distributors. For use in the investigation all the samples were ground to pass a 100-mesh sieve. With one exception (Collaborator 19) complete sets of the slags were not issued to the collaborators because of the large number of samples and the short supply of some of them.

Open-Hearth Slag, with Fluorspar.—Samples 1 and 2 are from materials made at two plants in the United Kingdom in late 1947 or early 1948. Sample No. 3 is a tapping slag produced at Sydney, Nova Scotia, in or about 1930.

TABLE 1.—*Samples for collaborative study of solubility of basic slag phosphorus in neutral ammonium citrate and 2 per cent citric acid solutions*

SAMPLE	TYPE AND SOURCE OF SLAG	TOTAL P ₂ O ₅		FLUORINE ^b
		RANGE ^a	AVERAGE	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	Open hearth, with fluorspar:			
1	United Kingdom	8.78–10.37	9.40	1.00
2	United Kingdom	11.00–11.82	11.33	0.58
3	Canada	10.67–11.30	11.00	1.50
	Open hearth, without fluorspar:			
4	United Kingdom	7.15– 7.96	7.62	0.08
5	United Kingdom	10.64–11.12	10.91	0.27
6	United Kingdom	10.78–11.88	11.44	0.11
7	United Kingdom	12.97–14.71	14.11	0.11
8	United Kingdom	14.60–15.72	15.11	0.09
	Open hearth:			
9	Alabama	8.09– 8.73	8.45	0.37
10	Alabama	8.20– 8.98	8.64	0.33
11	Alabama	11.48–12.09	11.86	0.14
	Bessemer:			
12	Europe	15.52–16.67	16.17	0.05
13	United Kingdom	16.18–17.07	16.60	0.09
14	Europe	17.67–18.25	17.98	0.10

^a Averages of replicate determinations on each sample by 11 collaborators.^b The analyses were made by E. J. Fox of this Bureau.TABLE 2.—*Mechanical composition of slag samples as received from the manufacturers and distributors*

SAMPLE	COMPOSITION, MESH ^a						
	+35	–35, +60	–60, +80	–80, +100	–100, +150	–150, +200	–200
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	4.6	9.8	8.6	8.4	9.8	13.6	45.2
2	5.2	2.6	6.0	6.8	10.8	14.8	53.8
4	6.0	3.6	4.0	4.6	9.0	17.0	55.8
5	1.2	3.8	4.4	4.4	7.6	14.6	64.0
6	1.2	3.8	5.4	5.4	9.2	14.6	60.8
7	2.8	6.2	6.6	6.6	9.6	13.8	54.4
8	2.4	6.4	6.4	6.2	8.6	13.6	56.4
10	0.2	1.4	1.4	3.0	7.0	14.0	73.0
11	13.8	7.2	4.4	4.6	6.6	10.6	52.8
13	0.6	3.8	4.4	4.4	6.8	11.0	69.0
14	3.0	1.2	1.4	2.5	5.2	11.6	75.1

^a Screen openings in sieve series were 420, 250, 177, 149, 104, and 74 microns, respectively.

Open-Hearth Slag, without Fluorspar.—These samples (Nos. 4–8), from three plants in the United Kingdom, are from materials produced in late 1947 or early 1948.

Open-Hearth Slag from Alabama.—Commercial production of basic slag in the United States is confined to the Birmingham, Alabama, area. Samples 9 and 10 are from materials made prior to 1942, while Sample 11 is from slag produced in early 1948.

Bessemer Slag.—Samples 12 and 14 are materials manufactured in Europe prior to 1940. Sample 13 was produced in the United Kingdom in late 1947 or early 1948.

COLLABORATORS' DIRECTIONS FOR ANALYSIS

1. Determine total P_2O_5 by the volumetric method as directed in *Methods of Analysis*, A.O.A.C., 1945, p. 23, sec. 2.12(a) or (b). Prepare the solution as directed on pp. 21–22, sec. 2.8(b).

2. Determine citrate-insoluble P_2O_5 as directed on p. 24, sec. 2.16(b). Dissolve the citrate-insoluble residue as directed in sec. 2.8(b) and determine P_2O_5 as directed in sec. 2.12(a) or (b).

3. Repeat the determinations of citrate-insoluble P_2O_5 as follows: Proceed as directed in sec. 2.16(b) through the point where the flask is first shaken vigorously to reduce the filter paper to a pulp. Next place the tightly stoppered flask in a continuous agitation apparatus provided with means for maintaining the contents of the flask at 65°C. and agitate for exactly 1 hour from the time the sample was introduced into the flask. Then proceed with the determination as before. Only those collaborators having access to continuous agitation, constant temperature devices are requested to determine citrate-insoluble P_2O_5 by the procedure outlined in this paragraph.

4. Prepare citric acid extracts of the samples as directed on p. 25, sec. 2.18, using one of the following modifications depending on the type of agitation apparatus available, and determine P_2O_5 as directed in sec. 2.12(a) or (b). It is important that the initial temperature of the citric acid solution be adjusted to 17.5°C.; that caking of the sample during addition of the citric acid solution be avoided; that the citric acid extract be filtered on a dry paper immediately after the digestion is completed; and that the clear extract be analyzed for P_2O_5 at once.

Modification I. Make the citric acid extraction with the aid of an end-over-end agitation apparatus (20–50 r.p.m.) and a wide-mouth, 250 ml. volumetric “fertilizer” flask, using a 2.5-gram sample, 2.5 ml. of alcohol, and sufficient citric acid solution to give a total flask content of 250 ml. As the 500-ml. Wagner flask specified in the official method is not commonly available in the fertilizer laboratories of the United States, this deviation from the official procedure is permissible because it has been shown (30) that with a constant ratio of weight of sample to volume of citric acid solution the results for citric acid-soluble P_2O_5 are not affected by variations in the sample weight. It has also been shown (28) that with end-over-end agitation in the range of 21–52 r.p.m. the results are not dependent on the speed of rotation of the flask.

Modification II. If an end-over-end agitation apparatus is not available, make the citric acid digestions (a) with the aid of continuous stirring or (b) with the use of a shaking apparatus such as the Ross-Kershaw machine or the Fisher “Gyrosolver.” In such cases, add 1 ml. of alcohol and 99 ml. of citric acid solution to 1 gram of the sample in a 250-ml. beaker for (a) or a 250-ml. “fertilizer” flask for (b).

5. Make all the determinations in triplicate, each on a separate portion of the sample, and report the individual results on the form enclosed with these Directions. If for any reason it is necessary to repeat a determination the repetition should be made in triplicate and the three results reported should be those obtained in simultaneous replications.

6. Your comments and observations concerning this investigation are requested.

COLLABORATORS

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PROCEDURES

For comparison with results by manual agitation at 5-minute intervals, eight of the collaborators determined citrate-insoluble P_2O_5 with the aid of continuous agitation, constant-temperature devices. For this purpose Collaborators 1, 10, 13, 15, 18, and 19 used the end-over-end rotation (20–22 r.p.m.) apparatus described by MacIntire, Marshall, and Meyer (29). Collaborator 3 used continuous stirring at 1,000 r.p.m., while Collaborator 11 employed an apparatus that moved the flask in a straight line in the horizontal plane at 70 oscillations per minute.

With the exception of Collaborator 18 who omitted this analysis, the determinations of citric acid-soluble P_2O_5 were made with the aid of

several types of continuous agitation devices as listed in Table 3, which also shows the weight of sample used in the extractions.

RESULTS OF ANALYSES

The results for citrate-insoluble P_2O_5 as determined respectively with the aid of continuous agitation and of manual shaking at 5-minute inter-

TABLE 3.—*Type of continuous agitation apparatus and weight of sample used in determining citric acid-soluble P_2O_5*

COLLABORATOR	APPARATUS		WEIGHT OF SAMPLE ^a
	TYPE	REVOLUTIONS OF OSCILLATIONS PER MINUTE	
			grams
1	End-over-end ^b	22	2.5
10	End-over-end ^b	21	2.5
13	End-over-end ^b	21	1.0
15	End-over-end ^b	22	2.5
19	End-over-end ^b	22	2.5
2	End-over-end	32	5.0
7	End-over-end	30	2.0
11	End-over-end	18	1.0
14	End-over-end	20	2.5
21	End-over-end	30	5.0
3	Stirring ^c	300	1.0
4	Stirring ^d	670	1.0
6	Stirring	650	1.0
8	Stirring ^e	250	1.0
17	Stirring	275	1.0
20	Stirring	360	1.0
9	Ross-Kershaw shaker ^{f,g}	120	1.0
12	Ross-Kershaw shaker ^f	180	1.0
16	Fisher "Gyrosolver" ^h	200	1.0
5	Kahn-type shaker ⁱ	165	2.5

^a In all cases the ratio of sample weight to solvent volume was 1 gram per 100 ml

^b MacIntire-Marshall-Meyer type (29); Catalog No. 5960, Precision Scientific Co., Chicago, Ill

^c To prevent caking, the contents of the beaker were stirred by hand preliminary to the continuous agitation.

^d Stirred with rod, 6 mm diameter with a right-angle bend 22-23 mm long, reaching close to bottom of beaker. The contents of the beaker were stirred by hand while adding the citric acid solution to the sample, and the beaker was turned in a 90° arc at 5-minute intervals during the continuous agitation period.

^e Stirred with straight rod revolving in a circle of 13 mm radius

^f Eccentric rotary movement in the horizontal plane (38); Catalog No. 30873, Eimer & Amend, New York, N. Y., 1936.

^g Flask was shaken by hand at 10-minute intervals during the continuous agitation period.

^h Movement in 3 planes; Catalog No. 14-258, Fisher Scientific Co., Pittsburgh, Pa., 1942.

ⁱ Straight-line oscillation in the horizontal plane; Catalog No. 8926, Arthur H. Thomas Co., Philadelphia, Pa., 1931. Flask was shaken by hand at 5-minute intervals during the continuous agitation period.

vals during the citrate digestion are shown in Table 4. Table 5 summarizes the results for citric acid-insoluble P_2O_5 and citrate-insoluble P_2O_5 , the latter determined with manual shaking at 5-minute intervals

TABLE 4.—*Effect of continuous agitation during citrate digestion on results for citrate-insoluble P_2O_5*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS ^c
	INTERMITTENT AGITATION ^a		CONTINUOUS AGITATION ^b		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	per cent	per cent	per cent	per cent
	Sample 1				
1	6.80–6.85	6.82	6.55–6.65	6.60	0.22
15	5.73–6.28	6.04	6.03–6.05	6.04	0.00
19	6.92–6.99	6.96	6.48–6.61	6.55	0.41
Group	5.73–6.99	6.61	6.03–6.65	6.40	0.21**
	Sample 2				
3	8.12–8.16	8.15	8.00–8.16	8.09	0.06
10	7.92–7.98	7.95	7.86–7.98	7.92	0.03
11	7.60–7.68	7.63	7.68–7.82	7.77	–0.14
13	7.70–7.80	7.77	7.60–7.75	7.68	0.09
18	7.86–7.96	7.91	7.88–8.05	7.94	–0.03
19	8.11–8.31	8.20	8.07–8.10	8.09	0.11
Group	7.60–8.31	7.94	7.60–8.16	7.92	0.02
	Sample 3				
15	8.55–8.70	8.62	9.10–9.10	9.10	–0.48
19	9.11–9.39	9.23	8.71–9.00	8.86	0.37
Group	8.55–9.39	8.92	8.71–9.10	8.98	–0.06
	Sample 4				
3	0.68–0.72	0.71	0.68–0.72	0.71	0.00
10	0.67–0.79	0.75	0.83–0.94	0.90	–0.15
11	0.88–0.92	0.89	0.58–0.78	0.71	0.18
13	0.90–0.95	0.93	0.85–0.90	0.88	0.05
18	0.68–0.73	0.70	0.73–0.77	0.75	–0.05
19	0.81–0.81	0.81	0.78–0.82	0.80	0.01
Group	0.67–0.95	0.80	0.58–0.94	0.79	0.01
	Sample 5				
15	0.58–0.67	0.63	0.78–0.82	0.80	–0.17
19	1.31–1.40	1.35	1.19–1.23	1.21	0.14
Group	0.58–1.40	0.99	0.78–1.23	1.00	–0.01
	Sample 6				
1	1.30–1.30	1.30	1.15–1.20	1.17	0.13
15	0.72–0.73	0.73	0.57–0.62	0.59	0.14
19	0.97–1.16	1.05	1.12–1.16	1.13	–0.08
Group	0.72–1.30	1.03	0.57–1.20	0.96	0.07*

TABLE 4—(continued)

COLLABORATOR	CITRATE-INSOLUBLE P ₂ O ₅ BY—				DIFFERENCE IN AVERAGE RESULTS ^c
	INTERMITTENT AGITATION ^a		CONTINUOUS AGITATION ^b		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	per cent	per cent	per cent	per cent
			Sample 7		
1	1.45–1.50	1.48	1.25–1.25	1.25	0.23
15	1.15–1.16	1.16	0.68–0.75	0.71	0.45
19	1.44–1.48	1.46	1.12–1.27	1.17	0.29
Group	1.15–1.50	1.36	0.68–1.27	1.04	0.32***
			Sample 8		
3	1.16–1.20	1.19	1.04–1.12	1.07	0.12
10	1.28–1.36	1.33	1.09–1.24	1.17	0.16
11	1.24–1.28	1.26	1.18–1.28	1.24	0.02
13	1.30–1.65	1.48	1.20–1.25	1.22	0.26
18	1.20–1.28	1.24	1.07–1.15	1.11	0.13
19	1.32–1.38	1.35	0.96–1.07	1.01	0.34
Group	1.16–1.65	1.31	0.96–1.28	1.14	0.17***
			Sample 9		
3	3.08–3.12	3.09	3.12–3.16	3.13	–0.04
10	3.22–3.27	3.25	3.30–3.40	3.35	–0.10
11	3.16–3.20	3.17	3.06–3.06	3.06	0.11
13	3.05–3.30	3.20	3.10–3.20	3.17	0.03
18	3.22–3.24	3.23	3.20–3.23	3.21	0.02
19	2.99–3.11	3.05	3.01–3.12	3.06	0.01
Group	2.99–3.30	3.16	3.01–3.40	3.16	0.00
			Sample 10		
1	2.25–2.30	2.28	2.20–2.20	2.20	0.08
15	1.84–1.89	1.87	1.89–1.97	1.92	–0.05
19	2.18–2.23	2.21	2.01–2.18	2.10	0.11
Group	1.84–2.30	2.12	1.89–2.20	2.07	0.05*
			Sample 11		
3	1.48–1.52	1.49	1.44–1.48	1.45	0.04
10	1.58–1.68	1.61	1.50–1.71	1.63	–0.02
11	1.60–1.64	1.63	1.32–1.36	1.34	0.29
13	1.40–1.60	1.50	1.50–1.55	1.52	–0.02
18	1.55–1.60	1.58	1.46–1.50	1.48	0.10
19	1.55–1.57	1.56	1.49–1.73	1.61	–0.05
Group	1.40–1.68	1.56	1.32–1.73	1.50	0.06*
			Sample 12		
1	1.70–1.75	1.73	1.50–1.50	1.50	0.23
15	1.40–1.46	1.42	1.36–1.37	1.37	0.05
19	1.67–1.69	1.68	1.55–1.67	1.60	0.08
Group	1.40–1.75	1.61	1.36–1.67	1.49	0.12***

TABLE 4—(continued)

COLLABORATOR	CITRATE-INSOLUBLE P ₂ O ₅ BY—				DIFFERENCE IN AVERAGE RESULTS ^c
	INTERMITTENT AGITATION ^a		CONTINUOUS AGITATION ^b		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	per cent	per cent	per cent	per cent
Sample 13					
3	1.80–1.80	1.80	1.80–1.84	1.81	–0.01
10	1.95–1.98	1.97	1.80–1.98	1.91	0.06
11	1.92–2.16	2.05	1.84–1.88	1.86	0.19
13	1.70–1.85	1.78	1.75–1.85	1.80	–0.02
18	1.84–1.87	1.86	1.74–1.80	1.78	0.08
19	1.66–1.85	1.75	1.82–1.83	1.83	–0.08
Group	1.66–2.16	1.87	1.74–1.98	1.83	0.04
Sample 14					
3	1.60–1.64	1.63	1.56–1.60	1.57	0.06
10	1.62–1.68	1.64	1.65–1.83	1.76	–0.12
11	1.48–1.60	1.53	1.48–1.56	1.53	0.00
13	1.55–1.70	1.63	1.60–1.70	1.65	–0.02
18	1.69–1.74	1.71	1.55–1.60	1.57	0.14
19	1.55–1.78	1.67	1.52–1.76	1.61	0.06
Group	1.48–1.78	1.63	1.48–1.83	1.61	0.02
Group Averages					
d	—	2.92	—	2.85	0.07 ^e
f	—	3.23	—	3.13	0.10 ^e
g,h	—	2.61	—	2.56	0.05 ^e
g,i	—	2.62	—	2.59	0.03 ^e
g,j	—	2.58	—	2.55	0.03 ^e
g,k	—	2.59	—	2.50	0.09 ^e

^a Manual shaking at 5-minute intervals during the citrate digestion^b Collaborators 1, 10, 13, 15, 18, and 19 used end-over-end agitation by the MacIntire-Marshall-Meyer apparatus; Collaborator 3 used continuous stirring at 1,000 r.p.m.; and Collaborator 11 used continuous shaking in a straight horizontal plane at 70 oscillations per minute^c The minus sign denotes that the result by continuous agitation is higher than that by intermittent agitation. Tests for significant differences were made only on the group results. One, two, and three asterisks denote that the differences are significant at the 5%, 1%, and 0.1% levels, respectively.^d All results.^e Not analyzed statistically.^f Samples 1, 3, 5, 6, 7, 10, 12.^g Samples 2, 4, 8, 9, 11, 13, 14.^h All collaborators.ⁱ Collaborators 1, 10, 13, 15, 18, 19; continuous agitation by end-over-end rotation.^j Collaborator 3; continuous agitation by stirring.^k Collaborator 11; continuous agitation by straight-line oscillation in the horizontal plane.

during the citrate digestion. For each collaborator and each sample the citric acid-insoluble P_2O_5 values were computed individually from the reported replicate results for citric acid-soluble P_2O_5 and the collaborator's average result for total P_2O_5 in the sample.

TABLE 5.—Citrate-insoluble and citric acid-insoluble P_2O_5 in basic slags

COLLABORATOR	CITRATE-INSOLUBLE $P_2O_5^a$		CITRIC ACID-INSOLUBLE $P_2O_5^b$		DIFFERENCE IN AVERAGE RESULTS ^c
	RANGE	AVERAGE	RANGE	AVERAGE	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
			<i>Sample 1</i>		
1	6.80–6.85	6.82	6.45– 6.50	6.48	0.34***
4	6.55–6.61	6.58	6.27– 6.32	6.29	0.29***
5	6.55–6.55	6.55	7.35– 7.39	7.36	–0.81 ^d
6	6.17–6.18	6.18	5.71– 5.86	5.79	0.39***
7	6.77–6.85	6.81	5.88– 5.93	5.90	0.91***
8	6.60–6.70	6.67	6.42– 6.92	6.63	0.04
12	6.52–6.61	6.56	6.34– 6.36	6.35	0.21**
14	6.97–7.10	7.02	5.74– 5.75	5.75	1.27***
15	5.73–6.28	6.04	7.07– 7.12	7.10	–1.06 ^d
17	6.40–6.66	6.54	6.55– 6.62	6.59	–0.05
19	6.92–6.99	6.96	5.87– 6.13	5.99	0.97***
Group ^e	6.17–7.10	6.68	5.71– 6.92	6.20	0.48***
Group ^f	5.73–7.10	6.61	5.71– 7.39	6.38	0.23*
			<i>Sample 2</i>		
2	7.95–8.02	8.00	6.82– 6.97	6.87	1.13***
3	8.12–8.16	8.15	7.13– 7.17	7.16	0.99***
9	7.68–8.10	7.90	7.56– 7.71	7.61	0.29***
10	7.92–7.98	7.95	7.08– 7.18	7.13	0.82***
11	7.60–7.68	7.63	7.29– 7.35	7.32	0.31***
13	7.70–7.80	7.77	7.35– 7.40	7.38	0.39***
16	7.95–8.08	8.03	7.38– 7.51	7.45	0.58***
19	8.11–8.31	8.20	6.97– 7.13	7.04	1.16***
20	8.21–8.25	8.22	7.72– 7.77	7.75	0.47***
21	8.17–8.20	8.18	7.77– 7.85	7.82	0.36***
Group ^f	7.60–8.31	8.00	6.82– 7.85	7.35	0.65***
			<i>Sample 3</i>		
4	9.23–9.27	9.25	8.88– 8.97	8.92	0.33***
5	9.28–9.30	9.29	10.36–10.38	10.37	–1.08 ^d
6	9.75–9.85	9.78	9.27– 9.47	9.35	0.43***
7	9.55–9.75	9.63	9.14– 9.21	9.18	0.45***
8	9.30–9.60	9.47	8.87– 8.95	8.91	0.56***
12	8.14–8.54	8.31	9.50– 9.60	9.55	–1.24 ^d
14	9.75–9.78	9.77	8.88– 8.91	8.89	0.88***
15	8.55–8.70	8.62	8.96– 8.96	8.96	–0.34***
17	9.45–9.64	9.55	9.46– 9.52	9.49	0.06
19	9.11–9.39	9.23	9.21– 9.52	9.34	–0.11
Group ^h	8.55–9.85	9.41	8.87– 9.52	9.13	0.28***
Group ^f	8.14–9.85	9.29	8.87–10.38	9.30	–0.01*
			<i>Sample 4</i>		
2	0.75–0.80	0.77	–0.03– 0.07	0.04	0.73***
3	0.68–0.72	0.71	0.25– 0.29	0.28	0.43***
9	0.68–0.74	0.70	0.88– 0.98	0.91	–0.21***

TABLE 5—(continued)

COLLABORATOR	CITRATE-INSOLUBLE $P_2O_5^a$		CITRIC ACID-INSOLUBLE $P_2O_5^b$		DIFFERENCE IN AVERAGE RESULTS ^c
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	per cent	per cent	per cent	per cent
10	0.67-0.79	0.75	0.51- 0.58	0.56	0.19***
11	0.88-0.92	0.89	0.53- 0.77	0.67	0.22***
13	0.90-0.95	0.93	1.03- 1.08	1.05	-0.12**
16	0.58-0.70	0.65	0.20- 0.35	0.26	0.39***
19	0.81-0.81	0.81	0.51- 0.66	0.57	0.24***
20	0.79-0.80	0.79	0.67- 0.73	0.70	0.09*
21	0.78-0.86	0.81	0.69- 0.86	0.75	0.06
Group ^f	0.58-0.95	0.78	-0.03- 1.08	0.58	0.20***
			Sample 5		
4	1.13-1.22	1.18	0.74- 0.78	0.75	0.43***
5	0.98-1.02	1.00	2.62- 2.67	2.65	-1.65 ^d
6	1.22-1.26	1.24	0.75- 1.05	0.89	0.35***
7	1.05-1.12	1.10	0.61- 0.69	0.65	0.45***
8	2.07-2.10	2.08	1.94- 2.52	2.16	-0.08
12	1.01-1.06	1.03	0.52- 0.57	0.54	0.49***
14	1.28-1.33	1.31	0.17- 0.19	0.18	1.13***
15	0.58-0.67	0.63	0.44- 0.49	0.46	0.17*
17	1.14-1.16	1.15	0.80- 0.92	0.84	0.31***
19	1.31-1.40	1.35	0.54- 0.61	0.58	0.77***
Group ^f	0.58-2.10	1.23	0.17- 2.52	0.78	0.45***
Group ^f	0.58-2.10	1.21	0.17- 2.67	0.97	0.24 ^e
			Sample 6		
1	1.30-1.30	1.30	0.67- 0.72	0.70	0.60***
4	1.19-1.27	1.23	0.61- 0.65	0.62	0.61***
5	0.86-0.91	0.89	3.12- 3.22	3.17	-2.28 ^d
6	1.12-1.18	1.15	1.05- 1.27	1.15	0.00
7	1.10-1.12	1.11	0.67- 0.71	0.69	0.42***
8	1.20-1.40	1.30	1.77- 2.57	2.10	-0.80 ^d
12	0.88-0.96	0.91	0.38- 0.41	0.39	0.52***
14	1.28-1.36	1.32	0.50- 0.69	0.59	0.73***
15	0.72-0.73	0.73	-0.02- 0.03	-0.02	0.75***
17	1.11-1.16	1.13	0.82- 0.95	0.88	0.25***
19	0.97-1.16	1.05	0.93- 1.03	0.97	0.08
Group ^f	0.72-1.36	1.10	-0.02- 1.27	0.66	0.44***
Group ^f	0.72-1.40	1.10	-0.02- 3.22	1.02	0.08 ^e
			Sample 7		
1	1.45-1.50	1.48	1.80- 1.85	1.82	-0.34
4	1.16-1.20	1.18	0.51- 0.64	0.55	0.63***
5	1.60-1.62	1.61	3.50- 3.58	3.54	-1.93 ^d
6	1.38-1.44	1.41	1.25- 1.46	1.37	0.04
7	1.27-1.32	1.30	0.77- 0.81	0.80	0.50**
8	1.40-2.00	1.73	-0.33- 1.12	0.15	1.58***

TABLE 5—(continued)

COLLABORATOR	CITRATE-INSOLUBLE $P_2O_5^a$		CITRIC ACID-INSOLUBLE $P_2O_5^b$		DIFFERENCE IN AVERAGE RESULTS ^c
	RANGE	AVERAGE	RANGE	AVERAGE	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
12	1.29-1.43	1.37	0.43- 0.53	0.50	0.87***
14	1.59-1.66	1.63	0.49- 0.54	0.52	1.11***
15	1.15-1.16	1.16	0.29- 0.29	0.29	0.87***
17	1.14-1.28	1.21	1.52- 1.76	1.64	-0.43*
19	1.44-1.48	1.46	0.84- 0.90	0.87	0.59**
Group ¹	1.14-1.66	1.39	-0.33- 1.85	0.85	0.54***
Group ^f	1.14-1.66	1.41	-0.33- 3.58	1.09	0.32*
<i>Sample 8</i>					
2	1.30-1.35	1.33	0.50- 0.65	0.55	0.78***
3	1.16-1.20	1.19	0.76- 0.80	0.79	0.40***
9	0.90-1.00	0.93	1.89- 2.15	2.04	-1.11 ^d
10	1.28-1.36	1.33	0.63- 0.68	0.65	0.68***
11	1.24-1.28	1.26	0.50- 0.65	0.58	0.68***
13	1.30-1.65	1.48	1.92- 2.07	2.00	-0.52 ^d
16	1.17-1.27	1.20	0.48- 0.57	0.52	0.68***
19	1.32-1.38	1.35	0.86- 0.93	0.89	0.46***
20	1.40-1.45	1.42	0.76- 0.77	0.76	0.66***
21	1.30-1.60	1.47	1.03- 1.08	1.05	0.42***
Group ^k	1.16-1.60	1.32	0.48- 1.08	0.72	0.60***
Group ^f	0.90-1.65	1.29	0.48- 2.15	0.98	0.31*
<i>Sample 9</i>					
2	3.15-3.20	3.18	2.92- 2.92	2.92	0.26***
3	3.08-3.12	3.09	2.94- 3.02	2.97	0.12**
9	3.36-3.40	3.37	3.19- 3.26	3.21	0.16***
10	3.22-3.27	3.25	2.94- 3.09	2.99	0.26***
11	3.16-3.20	3.17	2.81- 2.87	2.84	0.33***
13	3.05-3.30	3.20	3.12- 3.22	3.17	0.03
16	3.02-3.10	3.06	2.79- 2.96	2.90	0.16***
19	2.99-3.11	3.05	2.85- 2.89	2.88	0.17*
20	3.06-3.08	3.07	3.03- 3.04	3.03	0.04
21	3.06-3.20	3.12	3.16- 3.18	3.17	-0.05
Group ^f	2.99-3.40	3.16	2.79- 3.26	3.01	0.15***
<i>Sample 10</i>					
1	2.25-2.30	2.28	2.13- 2.18	2.15	0.13**
4	2.04-2.04	2.04	1.81- 1.82	1.82	0.22***
5	2.10-2.15	2.13	2.88- 2.93	2.90	-0.77 ^d
6	2.23-2.26	2.25	2.13- 2.23	2.19	0.06
7	2.10-2.12	2.11	2.04- 2.08	2.05	0.06
8	1.20-1.30	1.25	1.69- 1.82	1.74	-0.49***
12	2.11-2.13	2.12	1.67- 1.89	1.81	0.31***
14	2.14-2.16	2.15	1.79- 1.86	1.82	0.33***
15	1.84-1.89	1.87	1.45- 1.60	1.52	0.35***

TABLE 5—(continued)

COLLABORATOR	CITRATE-INSOLUBLE $P_2O_5^a$		CITRIC ACID-INSOLUBLE $P_2O_5^b$		DIFFERENCE IN AVERAGE RESULTS ^c
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	per cent	per cent	per cent	per cent
17	2.00-2.03	2.02	2.00- 2.09	2.04	-0.02
19	2.18-2.23	2.21	1.76- 1.87	1.81	0.40***
Group ¹	1.20-2.30	2.03	1.45- 2.23	1.90	0.13***
Group ^f	1.20-2.30	2.04	1.45- 2.93	1.99	0.05*
<i>Sample 11</i>					
2	1.57-1.62	1.60	1.00- 1.10	1.05	0.55***
3	1.48-1.52	1.49	1.23- 1.35	1.28	0.21**
9	1.60-1.68	1.64	2.72- 3.02	2.87	-1.23 ^d
10	1.58-1.68	1.61	1.33- 1.38	1.36	0.25***
11	1.60-1.64	1.63	1.13- 1.18	1.15	0.48***
13	1.40-1.60	1.50	2.17- 2.32	2.25	-0.75 ^d
16	1.55-1.65	1.60	0.91- 0.96	0.93	0.67***
19	1.55-1.57	1.56	1.11- 1.65	1.42	0.14*
20	1.66-1.66	1.66	1.37- 1.38	1.38	0.28***
21	1.56-1.66	1.59	1.58- 1.65	1.62	-0.03
Group ^k	1.48-1.68	1.59	0.91- 1.65	1.27	0.32***
Group ^f	1.40-1.68	1.59	0.91- 3.02	1.53	0.06*
<i>Sample 12</i>					
1	1.70-1.75	1.73	2.83- 2.88	2.86	-1.13 ^d
4	1.56-1.57	1.56	1.34- 1.42	1.38	0.18***
5	1.88-1.94	1.91	5.92- 5.97	5.93	-4.02 ^d
6	1.48-1.52	1.49	0.66- 0.70	0.68	0.81***
7	1.95-1.95	1.95	1.39- 1.43	1.41	0.54***
8	1.70-2.00	1.83	1.38- 1.43	1.41	0.42***
12	1.69-1.69	1.69	1.48- 1.68	1.55	0.14**
14	1.75-1.82	1.79	1.21- 1.23	1.22	0.57***
15	1.40-1.46	1.42	0.70- 0.75	0.73	0.69***
17	1.48-1.48	1.48	1.67- 1.88	1.77	-0.29***
19	1.67-1.69	1.68	1.43- 1.48	1.45	0.23***
Group ¹	1.40-2.00	1.65	0.66- 1.88	1.29	0.36***
Group ^f	1.40-2.00	1.68	0.66- 5.97	1.85	-0.17*
<i>Sample 13</i>					
2	1.87-1.90	1.89	1.08- 1.18	1.13	0.76***
3	1.80-1.80	1.80	1.51- 1.67	1.62	0.18***
9	2.00-2.08	2.05	3.12- 3.85	3.51	-1.46 ^d
10	1.96-1.98	1.97	1.33- 1.48	1.43	0.54***
11	1.92-2.16	2.05	1.53- 1.68	1.61	0.44***
13	1.70-1.85	1.78	2.32- 2.72	2.47	-0.69 ^d
16	1.72-1.87	1.80	1.47- 1.55	1.50	0.30***
19	1.66-1.85	1.75	1.50- 1.52	1.51	0.24***
20	1.75-1.79	1.77	1.53- 1.55	1.54	0.23***

TABLE 5—(continued)

COLLABORATOR	CITRATE-INSOLUBLE $P_2O_5^a$		CITRIC ACID-INSOLUBLE $P_2O_5^b$		DIFFERENCE IN AVERAGE RESULTS ^c
	RANGE	AVERAGE	RANGE	AVERAGE	
21	per cent 1.94–2.06	per cent 1.99	per cent 1.86– 1.89	per cent 1.88	per cent 0.11*
Group ^k	1.66–2.16	1.88	1.08– 1.89	1.53	0.35***
Group ^l	1.66–2.16	1.89	1.08– 3.85	1.82	0.07*
<i>Sample 14</i>					
2	1.65–1.70	1.67	0.90– 0.95	0.92	0.75***
3	1.60–1.64	1.63	1.21– 1.29	1.24	0.39***
9	1.84–1.96	1.89	3.18– 5.31	4.29	–2.40 ^d
10	1.62–1.68	1.64	1.26– 1.34	1.30	0.34***
11	1.48–1.60	1.53	1.37– 1.37	1.37	0.16***
13	1.55–1.70	1.63	2.15– 2.35	2.27	–0.64 ^d
16	1.60–1.62	1.61	1.12– 1.17	1.15	0.46***
19	1.55–1.78	1.67	1.22– 1.34	1.27	0.40***
20	1.53–1.55	1.54	1.33– 1.36	1.35	0.19***
21	1.88–2.04	1.96	1.44– 1.57	1.50	0.46***
Group ^k	1.48–2.04	1.66	0.90– 1.57	1.26	0.40***
Group ^l	1.48–2.04	1.68	0.90– 5.31	1.67	0.01*
<i>Group Averages</i>					
m	—	2.99	—	2.61	0.38*
n	—	2.98	—	2.82	0.16*

^a Manual shaking at 5-minute intervals during citrate digestion.

^b Computed from the collaborator's results for total P_2O_5 and citric acid-soluble P_2O_5 .

^c The minus sign denotes that the value for citric acid-soluble P_2O_5 is higher than that for citrate-insoluble P_2O_5 . One, two, and three asterisks denote that the differences are significant at the 5%, 1%, and 0.1% levels, respectively.

^d Collaborator's results are not included in the statistical analysis.

^e Excluding Collaborators 5 and 15.

^f Including all collaborators.

^g Not analysed statistically.

^h Excluding Collaborators 5 and 12.

ⁱ Excluding Collaborator 5.

^j Excluding Collaborators 5 and 8.

^k Excluding Collaborators 9 and 13.

^l Excluding Collaborators 1 and 5.

^m Results included in the statistical analyses.

ⁿ All results.

COMMENTS OF COLLABORATORS

Collaborator 5.—With the room temperature and the initial temperature of the citric acid solution at 29°C. and 17.5°C., respectively, the temperature of the citric acid extract was 24°C. at the end of the 30-minute digestion period. No difficulty was experienced with caking of the samples during the citric acid digestion.

Collaborator 6.—More difficulty was experienced with Sample 1 than with the other samples, as regards dehydration of the silica in the hydrochloric-nitric acid digestion of the citrate-insoluble residues and of the original materials.

Collaborator 7.—Before adding the molybdate solution it was necessary to acidify the citric acid extract more strongly with nitric acid than the official method calls for. Without the additional acid the solution developed a green color and no pre-

precipitate was formed. This condition appeared to be unaffected by the interval between the citrate digestion and addition of the molybdate reagent. All the reported values were determined on extracts that were filtered and analyzed immediately after the 30-minute citric acid digestion. With an initial citric acid temperature of 17.5°C. the temperature of the extract was 30°C. at the end of the digestion period.

Collaborator 8.—The results are not as good as desired, as we have had very little previous experience with basic slags, but shortness of time did not permit additional determinations. After most of the work was finished we suspected the following possible errors.

1. In digesting the sample for determination of total P_2O_5 , separation of gelatinous silica interferes with solution of the sample and may account for some of the discrepancies.

2. In the continuous stirring for extraction of citric acid-soluble P_2O_5 , we conclude that a straight stirring rod does not properly disperse the sample throughout the liquid. A mass of coarse particles collects in the center of the beaker, where it remains almost stationary.

Collaborator 9.—For the citric acid digestion the Ross-Kershaw machine at 120 oscillations per minute does not provide sufficient agitation. Without additional manual shaking a firm cake of the material forms on the bottom of the flask. The citrate extracts of Samples 2 and 8 were difficult to filter and wash, requiring 1 hour and 3 hours, respectively.

Collaborator 10.—For determination of total P_2O_5 the samples were digested with 10 ml. of perchloric acid plus 5 ml. of nitric acid. It is the opinion of this collaborator that only one value for total P_2O_5 in a sample should be used. This would make the values for available P_2O_5 in the sample independent of variations in the results for total P_2O_5 obtained thereon by the different collaborators.

Collaborator 13.—In the determination of total P_2O_5 the samples were dissolved by means of hydrochloric and nitric acids, as directed. Higher values were obtained on solutions prepared with sulfuric and nitric acids and analyzed by the volumetric method with precipitation of the phosphomolybdate at 25–30°C. and subsequent continuous agitation. The results are as follows:

SAMPLE	TOTAL P_2O_5 BY DIGESTION WITH—	
	HCl-HNO ₃	H ₂ SO ₄ -HNO ₃
	<i>per cent</i>	<i>per cent</i>
2	11.00	11.82
4	7.53	7.81
8	15.12	15.48
9	8.37	8.66
11	11.82	12.07
12	16.47	16.90
14	17.90	18.16

Collaborator 15.—Considerable difficulty was experienced with the hydrochloric-nitric acid method of dissolving the sample for total P_2O_5 determination owing to the presence of much gelatinous silica which made filtration very tedious. In addition to the reported P_2O_5 values which were obtained with this method of solution, determinations were also made on solutions prepared with the aid of hydrofluoric and perchloric acids, whereby the silica was volatilized and the hydrofluoric acid was expelled by fuming the mixture in a platinum dish. The results are as follows:

SAMPLE	TOTAL P_2O_5 BY DIGESTION WITH—	
	HCl-HNO ₃	HF-HClO ₄
1	<i>per cent</i>	<i>per cent</i>
	10.40	8.55
	10.35	8.70
	10.35	8.65
3	10.98	9.95
	10.55	10.00
	11.05	10.40
5	10.65	9.85
	10.45	9.90
	10.83	10.00
6	10.15	10.90
	10.78	11.00
	11.40	11.10
7	13.59	14.00
	13.84	13.99
	14.10	14.05
10	8.10	8.65
	8.06	8.55
	8.45	8.55
12	15.27	15.75
	15.75	15.90
	15.93	15.80
56a*	—	32.77

* National Bureau of Standards sample of Tennessee phosphate rock. Certificate value for total P_2O_5 is 33.01%.

Collaborator 19.—Thorough washing of the citrate-insoluble residues was tedious, requiring 3 to 4 hours in some cases.

INTERPRETATION OF RESULTS

Intermittent Shaking vs. Continuous Agitation During Citrate Digestion.—In last year's report on P_2O_5 (25) it was concluded that, in comparison with manual shaking at 5-minute intervals, continuous agitation during the citrate digestion tends to give somewhat lower values for citrate-insoluble P_2O_5 in basic slag and other furnace-made phosphates. Although the results of the present investigation generally confirm this conclusion, the differences are statistically significant for only 7 of the 14 samples submitted to the collaborators (Table 4). Even so, the significant differences between the group results for citrate-insoluble P_2O_5 by intermittent and continuous agitation—all in the direction of lower values

by the latter procedure—are quite small; they range from 0.05 to 0.32 per cent of the sample or 0.6 to 2.2 per cent of the group-average total P_2O_5 . In these comparisons, significance was determined by the F test using the variance within replicates as the error term. When collaborator variance was included in the error term, the differences between group averages for intermittent and continuous agitation did not attain significance in a single case.

In agreement with previous results (24, 25, 29) the differences in the average values for citrate-insoluble P_2O_5 (Samples 2, 4, 8, 9, 11, 13, and 14) by intermittent and continuous agitation, respectively, are the same or nearly so (Table 4), whether the latter was by end-over-end rotation (Collaborators 1, 10, 13, 15, 18, and 19), stirring (Collaborator 3), or straight-line oscillation in the horizontal plane (Collaborator 11).

Citrate-Insoluble P_2O_5 vs. Citric Acid-Insoluble P_2O_5 .—As previously pointed out, the values for citric acid-insoluble P_2O_5 (Table 5) are computed from the reported results for total P_2O_5 and citric acid-soluble P_2O_5 . Consequently these values, unlike those for citrate-insoluble P_2O_5 , also reflect such discrepancies as may occur in the results for total P_2O_5 . Careful inspection of the detailed data, partly omitted from this report, indicates that this is not an important factor in the differences between the tabulated values for citrate-insoluble and citric acid-insoluble P_2O_5 . In most cases these differences reflect principally the disparity, usually small, in the ultimate solvent action of the two reagents on the slag P_2O_5 under the specific conditions of the determinations.

Previous work (17, 18, 23, 24, 25, 36, 37) has indicated that with the present official methods the percentage of citrate-insoluble P_2O_5 in a slag can be expected, in general, to exceed that of the citric acid-insoluble P_2O_5 . This is true of 113 (78 per cent) of the 145 individual comparisons listed in Table 5. For the other 32 comparisons, widely distributed among 13 samples, close study of the data leads to the conclusion that the reversal in the sign of the differences is due mostly to too high values for citric acid-insoluble P_2O_5 relative to citrate-insoluble P_2O_5 , rather than the contrary. A possible major cause of such discrepancy is agglomeration and caking of the slag particles during the early stages of the digestion—a condition that is apt to occur, especially with citric acid—and subsequent failure to effect thorough dispersion of the sample. On this basis and in view of the large preponderance of positive differences (citrate-insoluble values higher than citric acid-insoluble values) omission from the statistical analyses of the comparisons that show negative differences greater than 0.5 per cent is considered justifiable.

Statistical analyses were made of the data for each sample. Significance was determined by the F test using the variance within replicates as the error term. Collaborator variance was excluded. The statistically significant differences at the 5, 1, and 0.1 per cent levels are indicated by ap-

appropriate asterisks in the last column of Table 5. In 19 of the 145 comparisons the values for citric acid-insoluble P_2O_5 exceeded those for citrate-insoluble P_2O_5 by more than 0.5 per cent; these comparisons were excluded from the statistical analysis.

Among the 126 individual comparisons that were tested statistically, 109 show significant differences between the values for citrate-insoluble and citric acid-insoluble P_2O_5 . Only 13 of the comparisons show higher values for citric acid-insoluble P_2O_5 than for citrate-insoluble P_2O_5 , and in 7 of the 13 the differences are not statistically significant. The distribution of the individual comparisons as regards statistical significance is as follows:

COMPARISONS	NUMBER	FRACTION OF TOTAL
		<i>per cent</i>
Tested statistically	126	100
Statistically significant	109	86.5
5.0 % level	6	4.8
1.0 % level	8	6.3
0.1 % level	95	75.4

All the average results on the 14 samples show statistically significant differences at the 0.1 per cent level, and these differences are all in the direction of higher values for citrate-insoluble P_2O_5 . For 8 samples (Nos. 3, 4, 5, 6, 7, 8, 11, and 12) the collaborator averages for citric acid-insoluble P_2O_5 are significantly more variable than those for citrate-insoluble P_2O_5 . Of the other 6 samples all but No. 10 show this trend though not attaining significance.

For the 145 individual comparisons the differences between the respective values for citrate-insoluble and citric acid-insoluble P_2O_5 range from -4.02 to $+1.58$ per cent (Table 5). Positive differences (citrate-insoluble P_2O_5 greater than citric acid-insoluble P_2O_5), averaging 0.45 per cent of P_2O_5 , are shown by 112 of the comparisons. For all of the 126 comparisons that were analyzed statistically, including 13 that show negative differences (range -0.02 to -0.49 per cent, average -0.20 per cent), the differences average $+0.38$ per cent of P_2O_5 . Of the statistically tested comparisons, 88 (69.8 per cent) show differences of not more than 0.5 per cent of P_2O_5 , while only 6 (4.4 per cent) show differences of more than 1 per cent. The distribution of the differences is shown in the table on the next page.

The data of Table 4 indicate that use of continuous agitation during the citrate digestion may narrow the gap between the citrate-insoluble and the citric acid-insoluble P_2O_5 values.

For the individual samples the statistically analyzed comparisons show average P_2O_5 differences—all positive—ranging from 0.13 per cent (Sample

CITRATE-INSOLUBLE P_2O_5 MINUS CITRIC ACID-INSOLUBLE P_2O_5	COMPARISONS	
	NUMBER	FRACTION OF TOTAL
<i>per cent</i>		<i>per cent</i>
-4.02 to -0.50*	19	13.1
-0.49 to -0.25	5	3.5
-0.24 to 0.00	9	6.2
0.01 - 0.10	10	6.9
0.11 - 0.20	14	9.7
0.21 - 0.30	16	11.0
0.31 - 0.40	18	12.4
0.41 - 0.50	16	11.0
0.51 - 0.60	9	6.2
0.61 - 0.80	15	10.4
0.81 - 1.00	8	5.5
1.01 - 1.58	6	4.1
-4.02 to +1.58	145	100.0

* Not analyzed statistically.

10) to 0.65 per cent (Sample 2) and averaging 0.38 per cent. On the basis of total P_2O_5 the differences range from 1.5 per cent (Sample 10) to 5.7 per cent (Sample 2) and average 3.1 per cent; these differences are of the same order as those previously found with finely ground alpha phosphates (17, 27).

With the possible exception of the Kahn-type shaker the values for citric acid-insoluble P_2O_5 show no clearly defined effects of variations in the type of continuous agitation device or the speed of agitation (Tables 3 and 5). This agrees with the findings of MacIntire, Hardin, and Meyer (28).

There appears to be no definite relation between the difference in the citrate-insoluble and the citric acid-insoluble P_2O_5 of a slag and either its type, source, total P_2O_5 , or fluorine (Tables 1 and 5). As would be expected however, the high-fluorine slags (Samples 1, 2, and 3) show low solubilities in both neutral ammonium citrate and 2 per cent citric acid; this is because the P_2O_5 in these samples is present principally in the form of fluorapatite (2, 27).

Although this report is concerned chiefly with the differences in the respective values for citrate-insoluble and citric acid-insoluble P_2O_5 , rather than with the actual values themselves, it should be noted that for a given sample the actual values for these determinations, as well as those for total P_2O_5 , are generally less consistent than is desirable in studies pertaining to official methods of analysis.

EFFECT OF PARTICLE SIZE

Hoffmeister (19) reported that when samples of ground basic slag were separated into different particle sizes the chemical composition of the

fractions and the solubility of the P_2O_5 in Wagner's acid ammonium citrate solution varied with the particle size, the solubility increasing with increase in fineness. Popp (33) also showed that the chemical composition of the mechanical fractions of ground basic slag varies with the particle

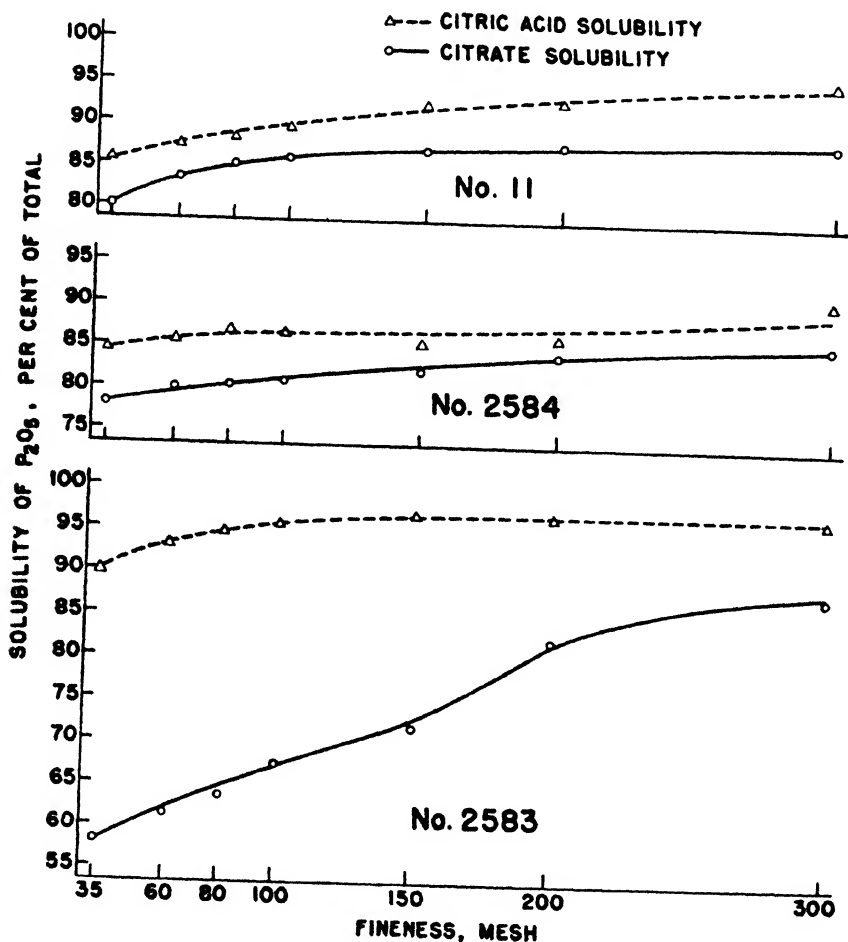


FIG. 1.—Effect of fineness on solubility of basic slag.

size; he reported, however, that the solubility of the P_2O_5 in 2 per cent citric acid did not vary greatly with the different fractions. Previous work in the writers' laboratory showed that the citrate and citric acid solubilities of a basic slag were not greatly affected by the fineness of the sample in the range —40 to —300 mesh (17, 18).

Solubility-fineness results obtained by the writers on three samples of slag are presented in Figure 1. Sample 11, an Alabama open-hearth slag, is

from one of the collaborative materials. Samples 2583 and 2584, containing 18.73 and 20.70 per cent of total P_2O_5 , respectively, are Bessemer slags recently manufactured in France. The fineness series of Samples 11 and 2584 were prepared from the commercially ground materials in such a way that all members had the same chemical composition as the original sample. Sample 2583 was received in the unground condition, and its fineness series was prepared in a manner similar to that for the other two samples.

As shown in Table 6 the fineness series of Samples 11 and 2584 have

TABLE 6.—*Mechanical composition of slags ground to different finenesses*

SAMP- LE	FINE- NESS*	COMPOSITION, MESH						
		-35, +60	-60, +80	-80, +100	-100, +150	-150, +200	-200, +300	-300
11	<i>Mesh</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	- 35	10.2	7.6	4.8	7.6	7.9	9.7	55.2
	- 60	—	10.2	6.3	8.2	9.2	10.6	55.5
	- 80	—	—	8.9	11.3	10.2	11.3	58.3
	-100	—	—	—	13.7	11.8	12.3	62.2
	-150	—	—	—	—	14.2	16.5	69.3
	-200	—	—	—	—	—	16.5	83.5
2583	- 35	25.0	20.4	10.8	13.7	9.4	7.3	13.4
	- 60	—	25.6	14.9	18.8	12.4	9.7	18.6
	- 80	—	—	21.4	25.0	16.0	12.2	25.4
	-100	—	—	—	32.0	20.1	15.5	32.4
	-150	—	—	—	—	28.7	23.1	48.2
	-200	—	—	—	—	—	48.4	51.6
2584	- 35	7.8	6.8	4.4	8.0	9.1	12.4	51.5
	- 60	—	7.7	5.3	8.9	10.1	13.2	54.8
	- 80	—	—	5.9	10.3	11.1	14.0	58.7
	-100	—	—	—	11.0	12.0	15.4	61.6
	-150	—	—	—	—	13.6	17.7	68.7
	-200	—	—	—	—	—	10.4	89.6

* Screen openings in sieve series were 420, 250, 177, 149, 104, 74, and 46 microns, respectively.

about the same distribution of particle size in the corresponding members, while Sample 2583 has considerably higher percentages of material in the coarser fractions. The determinations were made by sieving 10-gram samples, previously dried at 100°C., for 30 minutes in a Ro-Tap machine on U. S. standard screens that had been warmed to hinder condensation of moisture during the analysis. Because of extensive agglomeration of the finer particles in the presence of small amounts of moisture, the use of undried samples and cold screens during summer periods of high atmospheric humidity, as was the case at the time this solubility-fineness

study was made, may easily lead to erroneous particle-size distribution values in the screen series finer than 150 mesh. This is illustrated by the following results which were obtained by sieving 10-gram portions of 150-mesh material (Sample 11) in a Ro-Tap machine for 30 minutes:

CONDITIONS	COMPOSITION, MESH		
	-150, +200	-200, +300	-300
Undried sample, cold screens	<i>per cent</i> 15.3	<i>per cent</i> 55.9	<i>per cent</i> 28.8
Dry sample, warmed screens	14.2	16.5	69.3

The solubility-fineness data for Samples 11 and 2584 (Fig. 1) indicate that with slags commercially ground to a fineness of 85 per cent or more through a 35-mesh screen the gap between the citrate- and the citric acid-solubility values is narrowed but little, if any, by regrinding the analytical samples completely to pass screens finer than 35 mesh. For such samples, a rigid interpretation of the results points, however, to a fineness of -200 to -300 mesh in order to reduce the particle-size effect on the solubility to negligible values. Nevertheless, a fineness of -100 mesh sacrifices only 2 to 5.5 per cent of the P_2O_5 to the insoluble fraction.

That variation of the particle-size distribution in the fineness series has a much smaller effect on the citric acid solubility of slag P_2O_5 than on its citrate solubility is indicated by the data for Sample 2583. Especially as regards citrate solubility, this situation raises the question of particle-size distribution and its reproducibility in the preparation of slag samples for analysis, which apparently has an important bearing on analytical variability when the fineness of the sample does not lie in the range of a nearly horizontal portion of the solubility-fineness curve. The limited evidence (Fig. 1 and Table 6) indicates that for citrate solubility the slag fineness corresponding to the nearly horizontal portion of the solubility-fineness curve is 100 per cent through a 100-mesh screen and at least 95, 75, and 60 per cent through 150-, 200-, and 300-mesh screens, respectively.

SUMMARY AND CONCLUSIONS

In last year's report (25) it was shown for basic slag and a wide variety of other phosphatic materials (a) that the results for citrate-insoluble P_2O_5 by continuous agitation during the citrate digestion differ only slightly from those by shaking at 5-minute intervals and (b) that variation in the method of continuous agitation is not an important factor. The current work with basic slag supports these findings.

Triplicate determinations of citrate-insoluble and citric acid-insoluble P_2O_5 in 14 samples of different types and sources of basic slags were made by 10-11 collaborators using the present official methods with manual

shaking at 5-minute intervals during the citrate digestion. The slags, all received in the commercially ground condition, were reground to pass a 100-mesh screen for analysis.

The values reported by the individual collaborators for citrate-insoluble P_2O_5 are usually higher than those for citric acid-soluble P_2O_5 in the same sample. However, for the individual samples the average differences are small though statistically significant. Excluding certain results that are obviously considerably out of line, they range from 0.13 to 0.65 per cent and average 0.38 per cent of P_2O_5 on the sample, or 1.5 to 5.7 per cent (average 3.1 per cent) of the total P_2O_5 , all in the direction of higher values for citrate-insoluble P_2O_5 .

The results of this study, together with those of previous investigations, point to the present official neutral ammonium citrate method as a suitable procedure for the evaluation of the P_2O_5 in basic slags.

CITRATE-SOLUBLE AND AVAILABLE PHOSPHORIC ACID

In the *Methods of Analysis*, 1940, p. 24, sec. 17, the following directions are given for obtaining citrate-soluble and available P_2O_5 :

"Subtract sum of water-soluble and citrate-insoluble P_2O_5 from total to obtain the citrate-soluble P_2O_5 . Subtract citrate-insoluble P_2O_5 from total to obtain available P_2O_5 ."

At the 1944 meeting of this Association it was recommended by the Referee on Fertilizers (7) and by Subcommittee A on Recommendations of Referees (10) that these directions be revised to change the last sentence to read, "Subtract citrate-insoluble P_2O_5 from the total to obtain chemically available P_2O_5 in dicalcium phosphate, precipitated bone phosphate, and precipitated bone" (official, final action). As printed in the *Methods of Analysis*, 1945, p. 25, sec. 2.17, this sentence is further changed to include "acidulated samples."

Thus, aside from the citric acid procedure for basic slag, there is at present no official method for the determination of available P_2O_5 in materials other than acidulated samples and the three so-called "non-acidulated" products—dicalcium phosphate, precipitated bone phosphate, and precipitated bone. Though the official citrate procedures make no mention of mixed fertilizers, the available P_2O_5 in such materials is commonly determined by the procedure for acidulated samples, regardless of the type of phosphate (acidulated or non-acidulated) present in the mixture. This situation leaves the manufacturer and the State fertilizer control authorities without an official method for available P_2O_5 in any other non-acidulated phosphatic material until such time as the Association may designate a specific method for that particular material. However, the material loses its identity when it is included in a mixed fertilizer, and its P_2O_5 content is then subject to evaluation by the procedure for acidulated samples. Furthermore there is uncertainty as to the specific

kinds of phosphatic materials and compounds, aside from mixed fertilizers, that are covered by the respective terms, "acidulated samples" and "non-acidulated samples."

In view of the fact that a number of investigations (1, 3, 4, 5, 6, 13, 18, 20, 22, 26, 31, 35, 36, 37), including several (26, 35, 36, 37) conducted under the auspices of this Association, have shown a close relation between the citrate solubility of the P_2O_5 of a wide variety of phosphatic materials and their effectiveness in promoting plant growth, when the citrate digestion is made with a ratio of 1 gram of sample per 100 ml. of solvent, it seems appropriate that the Association reconsider the present directions for obtaining citrate-soluble and available P_2O_5 , with the object of specifying the use of the neutral ammonium citrate procedure for the evaluation of all types of phosphatic materials until such time as it may be demonstrated to the satisfaction of the Association that other procedures are better adapted for specific materials. The results of the present study of basic slag lend further support to this suggestion.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation of the fine cooperation given by the collaborators and their respective organizations in the investigation covered by this report.

Most of the samples of basic slag were kindly supplied by B. P. Curtis, Tennessee Coal, Iron and Railroad Company, Birmingham, Alabama; J. G. Devys, American Industrial Development Corporation, New York, New York; E. P. Hudson, Scottish Agricultural Industries Limited, Edinburgh, Scotland; and M. H. R. J. Plusjé, Staatsmijnen in Limburg, Geleen, Netherlands.

RECOMMENDATIONS*

It is recommended—

(1) That in the *Methods of Analysis*, A.O.A.C., 1945, sec. 2.10(b) be changed by substituting "324.03" for "323.81" in line 1 and "32.40" for "32.38" in line 2 (final action).

(2) That sec. 2.11 be changed by deleting "and dilute to 200 ml with H_2O ," designating the paragraph as "(a)," and adding a second paragraph as follows:

"(b) *Not applicable in preparation of solns by sulfuric acid digestion.*—Proceed as directed under 2.8(a), (b), or (c), preferably (a) when these acids are a suitable solvent, to point where acid digestion of sample is completed. Add 25 ml of 10% $BaCl_2$ soln to the hot digestate, boil ca 2 min, and continue as directed under 2.8" (final action).

* For report of Subcommittee A and action by the Association, see *This Journal*, 32, 43 (1949).

(3) That sec. 2.12 be changed by:

- (1) Adding to sec. 2.12(a) the following as the first sentence:
"Prepare soln of sample as directed under 2.11(a)."
- (2) Deleting the phrase, in sec. 2.12(b) first line, "Not applicable to superphosphate and other fertilizers that contain sulfates (5)," and adding the following as the first sentence: "Prepare soln of sample as directed under 2.11(b)."
- (3) Adding to sec. 2.12 a new paragraph, as follows:
"(c) *Not applicable to superphosphate and other fertilizers that contain sulfate or to solns prepared with the aid of sulfuric acid (5).*—Prepare soln of sample as directed under 2.11(a). Proceed as directed under (b)" (final action).

(4) That the first six sentences of sec. 2.16(a), lines 1–11, be changed (final action) as follows:

"After removing water-soluble P_2O_5 , 2.13, transfer the filter and residue, within a period not to exceed 1 hour, to 200 or 250 ml flask containing 100 ml NH_4 citrate soln previously heated to 65°. Close flask tightly with a smooth rubber stopper, shake vigorously until filter paper is reduced to pulp, relieve pressure by momentarily removing stopper, and proceed by one of the following methods: (1) Loosely stopper flask to prevent evaporation, place in water bath regulated to maintain contents of flask at exactly 65°, keep level of H_2O in bath above that of citrate soln in flask, and shake every 5 min; (2) continuously agitate contents of stoppered flask by means of apparatus equipped to maintain contents of flask at exactly 65°. At expiration of exactly 1 hour from time filter and residue were introduced, remove flask from bath or apparatus and immediately filter contents as rapidly as possible thru Whatman filter paper No. 5 or other paper of equal speed and retentiveness."

(5) That sec. 2.16 be altered by:

- (1) Changing the phrase, in sec. 2.16(a) first line, "*Acidulated samples*" to "*Acidulated samples and mixed fertilizers.*"
- (2) Deleting the words, in sec. 2.16(b) first line, "*other than basic slag*" (first action).

(6) That sec. 2.17 be changed by deleting the words, in second sentence, "in acidulated samples, dicalcium phosphate, precipitated bone phosphate, and precipitated bone" (first action).

(7) That the methods for citric acid-soluble phosphoric acid in basic slag, sec. 2.18, 2.19, and 2.20, be deleted (first action).

(8) That work on methods for phosphoric acid be continued, with emphasis on:

- (1) Evaluation of sintered, fused, and calcined alpha phosphates as fertilizers.
- (2) Aging of the molybdate solution used in the volumetric method to determine if a time limit should be put on its use or an addition made to preserve it.

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REPORT ON MOISTURE IN FERTILIZERS

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The work on moisture this year covers the results of the collaborative study of methods ordered last year, and also a discussion of the distillation method for moisture in fertilizers, as well as requested suggestions as to needed editorial changes in the Book of Methods. These three topics and the recommendations comprise the subjects, respectively, of the four sections of this report.

SECTION A. COLLABORATIVE STUDY OF DETERMINATION OF MOISTURE IN FERTILIZERS BY OVEN-DRYING, VACUUM- DESICCATION, AND AIR-FLOW METHODS

This study was recommended and approved last year¹ for the second time. It marks the beginning of another organized effort to do something about the long-recognized need for a method that is more satisfactory than the present official procedure for determining moisture in fertilizers. A rather full discussion of the problems of moisture determination in fertilizers was presented before the Association a few years ago.²

Two points of view regarding moisture determination in fertilizers have been prominent during the past few years. The fertilizer analyst tends to view water in fertilizers merely as a diluent. He is interested mainly in a moisture determination that will enable him to reduce his analytical results to a common basis as regards water content. The determination of water for this purpose need not involve considerations as to the actual state of the water in the sample so long as the result includes all or a definite part of the total water. The fertilizer technologist or manufacturer, on the other hand, has a vital interest in the free water since this is

¹ *This Journal*, 31, 234 (1948); 31, 42 (1948).

² *This Journal*, 25, 132 (1942).

the part of the total water that so largely affects the physical condition of the fertilizer. But since the free water value can often be used to reduce the results of analysis to a standard base, a very strong case can be made for setting up a procedure for the rapid determination of free water and requiring its use as a general method for all fertilizer purposes. The principal obstacle to this approach is the condition that free water does not always stay put—under some circumstances free water today may in part be bound as water of crystallization tomorrow, in which cases the analyst needs to know the sum of the two interchangeable forms, or else condition the sample at a definite temperature. Thus, in order to meet the needs of all concerned, at least two general methods will be required—one for free water, and another for the sum of the free water and that bound as water of crystallization. Although methods known to fulfill the necessary requirements are not at hand, the realization that any search for a single method for determining water in fertilizers for all purposes holds little promise, is a first step toward the solution of the problem.

With this consideration as a background the recommendation has been pursued by having determinations by the three methods—oven drying at 100°C. (official), vacuum desiccation at room temperature, and air flow at 60°C—performed on six typical fertilizers and fertilizer materials in a dozen fertilizer laboratories and comparing the variabilities of the data with respect to collaborators, materials, and methods. The results thus obtained are presented in this section of the report.

MATERIALS AND METHODS

Descriptions of procedures, special apparatus and other details, except the samples, are given below in the instructions to collaborators. The samples were ground to pass a U. S. No. 30 standard sieve. Sample I,

TABLE 1.—*Formulation of mixed fertilizer samples*

INGREDIENT	AMOUNT OF INGREDIENT IN—		
	SAMPLE IV 10-6-4 MIXTURE ¹	SAMPLE V 10-10-10 MIXTURE ²	SAMPLE VI 5-10-5 MIXTURE ²
	<i>Lb. per ton</i>	<i>Lb. per ton</i>	<i>Lb. per ton</i>
Nitrogen solution IIA	—	200	140
Ammonium sulfate	330	278	96
Ammonium nitrate	300	200	67
Superphosphate	600	782	1012
Double superphosphate	—	100	—
Potassium chloride	140	330	160
Magnesian limestone	—	110	325
Cocoa shell meal	—	—	200
Soybean meal	630	—	—

¹ Laboratory preparation.

² Commercial fertilizer.

the reference sample, was potassium sulfate, reagent quality (A.C.S. Standard) that had stood for some time in a closed container after the addition of about 4 per cent of water by spraying. Sample II was run-of-pile superphosphate, without added conditioner of any kind, that had been stored in an air-tight container for 12 months. Sample III, cocoa shell meal, was received from a fertilizer concern in December 1947. Samples IV, V, and VI were mixed fertilizers; their formulations are shown in Table 1.

GENERAL INFORMATION AND INSTRUCTIONS FOR COLLABORATORS

1. The official samples will be shipped about January 14, 1948. Six samples are to be analyzed in triplicate for moisture by twenty collaborators with the use of three methods.

2. The methods are: (A) oven drying (official), (B) vacuum desiccation, and (C) air flow at 60°C. The procedures to be followed are given below. The higher temperature prescribed in the Book of Official and Tentative Methods for potassium salts is to be disregarded in this study.

3. A complete set of samples (18 bottles) consists of the following materials: I. Potassium sulfate, II. Ordinary superphosphate, III. Organic material, IV. Mixed fertilizer, 10-6-4 grade, V. Mixed fertilizer, 10-10-10 grade, and VI. Mixed fertilizer, 5-10-5 grade. There are three identical bottles of each material, which will enable the collaborator to segregate the bottles into *three identical sets of the six samples*. A single determination by each of the three methods is to be made on the contents of each bottle. **DO NOT BREAK THE SEAL ON A BOTTLE UNTIL READY TO WEIGH SAMPLES FROM ITS CONTENTS.**

4. Practice determinations shall be made on a suitable material obtainable in the analyst's laboratory. Then single determinations shall be made on one set of the official samples by the three methods with due regard for the order of weighing prescribed below. Because of the necessity for making the three weighings from a bottle in rapid succession, a set of single determinations on the contents of a bottle will need to be run simultaneously. It is not absolutely necessary that all materials be run simultaneously. Each of the other two sets of official samples shall be used in a like manner to obtain replicate results.

5. The result in percentage to two decimals for each single determination on the official samples shall be reported on the attached form. Only spills, mechanical failure, or other *known* sources of gross error afford sufficient justification for not reporting a result. If such loss of data should occur, the partial set of results shall be reported, and in addition, another set of determinations by the three methods shall be made on the same bottle and the results shown on the margin of the report.

6. The order of weighing from a bottle for determinations by the different methods has been randomized among the collaborators. You are requested always to allocate your weighings in the following order:

1st weighing to Method _____
 2nd weighing to Method _____
 3rd weighing to Method _____

The three weighings should follow one another as rapidly as the skill of the operator will permit. In case the analyst should desire for his own information to run duplicate determinations a second set of three weighings shall be made and allocated to the methods in the foregoing order. However, except in the case of accidental loss

of a determination, only results on the first set of weighings are to be included in the formal report.

7. Collaborators are respectfully urged to follow the instructions with great care in order to avoid the introduction of spurious variability into the assembled results and consequent impairment of the validity of the tests this study has been designed to provide. Comments on, and studied criticism of, both method of study and procedures will be appreciated.

PROCEDURES

Note on weighing technique:

It is requested that the analyst catch the first weight on adding the sample to the weighing dish without any attempt to adjust the weight of the sample to an exact predetermined value.

Method A. Official oven drying:

Heat 2 grams of sample for 5 hours in water oven at temperature of boiling water (98°–100°C.). Report percentage loss in weight as moisture.

Method B. Vacuum desiccation:

Place 2 grams of sample in a tared low-form weighing dish (4 grams of sample may be used with large weighing dishes, 1.5–2 inches in diameter) and place it in a vacuum desiccator at 25–30°C. over anhydrous magnesium perchlorate (or equivalent desiccant) for 16–18 hours under not less than 20 inches of vacuum. Report percentage loss in weight as moisture.

Method C. Air flow at 60°C.:

Place 2 grams of sample in a tared fritted glass crucible and seat the crucible on a manifold in the oven at 60°C. Aspirate for 2 hours under 15 inches of vacuum. Cool in a desiccator and report the percentage loss in weight as moisture.

The air-flow method is described in a recent article entitled "The Air-flow Method for the Determination of Moisture in Fertilizers."³ The necessary equipment consists of a constant-temperature oven, provided with a horizontally-disposed U-shaped manifold that is connected through a gage to a source of vacuum, and a supply of fritted glass crucibles. Any standard laboratory oven will suffice, but a type with vents so placed that the incoming air passes directly over the heating coils is to be preferred. The vacuum gage is a standard instrument.

A 6-crucible manifold can be readily assembled (Fig. 1) in the laboratory with the use of the following pipe fittings:

5 tees, $\frac{1}{2}" \times \frac{1}{2}" \times \frac{1}{4}"$
2 ells, $\frac{1}{2}" \times \frac{1}{4}"$
2 ells, $\frac{1}{2}"$
8 close nipples, $\frac{1}{2}"$
7 nipples, $\frac{1}{4}" \times 1\frac{1}{2}"$ long

Crucible seats are provided by placing No. 6, 1-hole rubber stoppers over the vertical $1\frac{1}{2}"$ nipples. The nipple should extend about $\frac{1}{8}"$ above the

³ *This Journal*, 30, 840 (1947).

stopper to facilitate centering of the crucible over the opening. All connections must be air-tight when the vacuum is on. To this end the lower edge of the crucible and the upper surface of the stopper must be ground smooth to insure a proper seal at this point.

The fritted glass crucibles are the Gooch type, Pyrex glass, 15-ml. capacity, with straight sides, 45 mm. high, 30 mm. in diameter at the top and 20 mm. in diameter at the bottom, with a fine-porosity disc 20 mm. in diameter. All crucibles in a set should have about the same porosity.

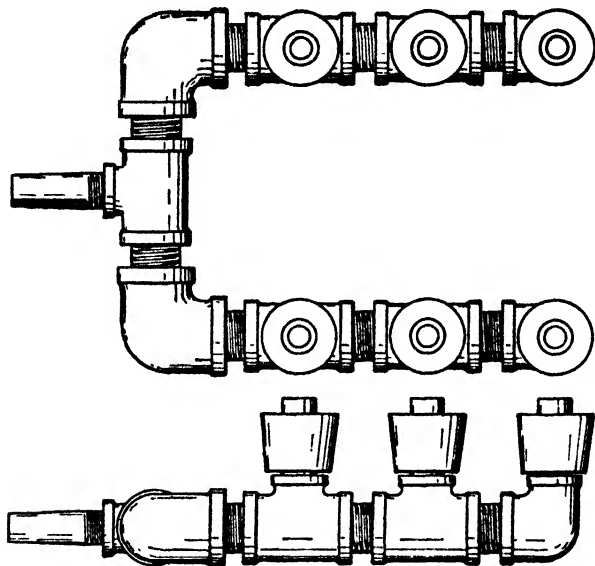


FIG. 1.—Views of manifold assembled with the use of pipe fittings.

A matched set is very desirable and can be rather simply obtained by selecting from the supply those that pass a given volume of air under constant pressure in approximately the same length of time.

COMMENTS OF COLLABORATORS

Samples.—"Bottles were too full to permit mixing before weighing out the sample."—C. A. Butt. "No difficulty was experienced in weighing samples with the exception of Sample No. 1, which was badly caked. Bottles were not too well sealed, and I would suggest that they be sealed with wax in future work."—C. T. McCloud.

Air-Flow Method.—"Our lot of 24 crucibles, when filled with water, varied in emptying times from 10 to 120 seconds."—J. B. Smith. "In setting up the air-flow apparatus we fitted a small piece of rubber tubing over the nipples, which provides a better seat than stoppers for the crucibles and also eliminates the possibility of the crucible toppling over when the vacuum is broken."—C. T. McCloud.

RESULTS AND THEIR VARIABILITY

Only twelve of the twenty-one laboratories that received sets of samples were able to perform the necessary analytical work. Two reports, re-

ceived in July, are excluded from the discussion, because, as was noted by another collaborator, the bottles were not too well sealed and certainly the closure was not adequate to carry them through the summer. The results from the ten remaining reports were obtained between January 30 and May 14—a period of 3.5 months, whereas it had been hoped that the work would all be done within a month.

TABLE 2.—*Collaborator and method means of results for percentage moisture in collaborative samples*

SAMPLE NO.	METHOD ^a	RANGE OF COLLABORATOR MEANS	METHOD MEAN		
			AVERAGE OF COLLABORATOR MEANS	STANDARD DEVIATION	COEF. OF VARIATION
I (Potassium sulfate)	A	2.60–4.02	3.57	0.473	13.25
	B	2.55–4.05	3.56	0.480	13.48
	C	2.76–4.02	3.56	0.467	13.12
II (Superphosphate)	A	5.33–6.92	6.34	0.482	7.60
	B	2.82–3.55	3.31	0.253	7.65
	C	3.66–4.70	4.20	0.328	7.80
III (Cocoa shell meal)	A	3.63–4.47	4.21	0.229	5.44
	B	0.22–2.51	1.52	0.710	46.7
	C	1.07–2.84	2.25	0.480	21.3
IV (10-6-4 fertilizer)	A	6.48–8.54	7.28	0.531	7.29
	B	3.98–4.74	4.54	0.233	5.14
	C	4.33–4.92	4.79	0.190	3.97
V (10-10-10 fertilizer)	A	1.43–2.37	1.75	0.276	15.77
	B	0.55–1.08	0.86	0.176	20.49
	C	0.70–1.19	0.99	0.148	14.94
VI (5-10-5 fertilizer)	A	4.78–6.13	5.29	0.365	6.90
	B	2.92–4.04	3.55	0.310	8.74
	C	3.45–4.21	3.90	0.229	5.88

^a The letters A, B, and C designate the oven-drying (official), vacuum-desiccation, and air-flow methods, respectively.

Ranges of collaborator means (averages of replicates) and method means (averages of collaborator means) are given in Table 2. The individual analyses, being unnecessary for the immediate discussion, are omitted from this report. The variability of the results by the three methods is rather high (Table 2), varies from sample to sample, and amounts to 0.176 to 0.710 in terms of standard deviation. The next highest standard deviation is 0.531, which compares favorably with that of a recent set of moisture analyses on a commercial check sample.⁴ The rela-

⁴ E. W. Magruder's check sample No. 1, January 1947, for which the average moisture was 4.05 per cent, standard deviation (omitting analysis 76) 0.51 and average P_2O_5 was 31.42 per cent, standard deviation 0.34.

tive variability of the results by the three methods is readily appreciated with the aid of coefficients of variation (standard deviation expressed in percentage of the method mean). Thus, considering numerical values of this coefficient without regard for significance, the variability of the results by the three methods were approximately the same on samples I and II. In the case of the cocoa shell meal (sample III) oven drying gave the most consistent results, despite the fact that this material is known to decompose in part at 100°C. In the remaining samples, all mixed fertilizers, the air-flow method gave the most consistent results.

SIGNIFICANCE OF RESULTS

In order to obtain a view of the structure of the variability and to test different effects for significance, the mean square variance of the results has been separated into four source factors— collaborators, methods, interaction of collaborators with methods, and replicates (Table 3). The statistical significance of the mean squares for the first three sources was determined by comparing them samplewise with those of the replicates. With the exception of the reference sample (I) the results are all very highly significant (odds 999:1).

TABLE 3.—*Variances contributed by certain sources of variability in moisture data*

VARIATION		MEAN SQUARES FOR SAMPLE NO.—					
SOURCE	DEGREES ^a OF FREEDOM	I	II	III	IV	V	VI
Collaborators ^b	9	1.98***	0.83***	1.60***	0.44***	0.28***	0.45***
Methods	2	0.0009	73.03***	58.00***	69.00***	6.91***	25.47***
C×M	18	0.018	0.19***	0.38***	0.34***	0.054***	0.20***
Within replicates	60	0.0552	0.0264	0.0263	0.0195	0.0175	0.0218

^a Total of 89.

^b Includes also sample variability, for example, change in moisture content with time either by reaction or by evaporation.

*** Significant, odds 999:1.

Collaborators.—The variance ascribed to collaborators covers, among other things, variability of sample, which admittedly was appreciable in some instances, and variability arising from the effect of atmospheric conditions on the sample during weighing and drying, which may differ widely between localities and vary markedly at the same location. The latter effect proved to be significant in four of the five instances in which this factor could be isolated. The collaborator variance (Table 3) is relatively large in the cases of samples II and III, which could be taken as an indication that these materials were susceptible to more rapid changes on exposure, especially the dried samples, than the other samples of the set. Measurements of weight gains on exposure of oven-dried samples (Fig. 2) show that absorption of atmospheric moisture could hardly ac-

count for the large collaborator variability in the case of sample II. At any rate, one could reasonably expect that rigid adherence by the analyst to the minute and tedious details of good weighing technique, both in taking the sample and in manipulating the dried sample, would markedly

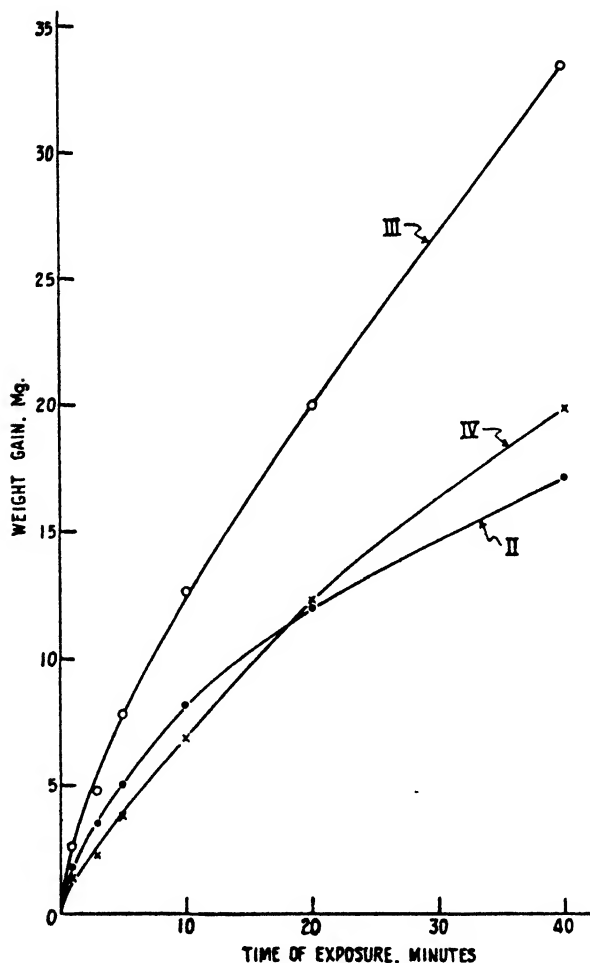


FIG. 2.—Increase in weight of oven-dried (105°) samples of superphosphate (II), cocoa shell meal (III) and a mixed fertilizer (IV) upon exposure to the atmosphere (25°C. and about 40% relative humidity).

reduce collaborator variability in the moisture determination by any method.

Methods.—The interaction of collaborator with methods ($C \times M$, Table 3) indicates merely that the collaborators were not equally skilled in the use of all three methods, provided they all had equally satisfactory equipment. The latter condition certainly was not realized in this study.

For example, some collaborators had difficulty with the maintenance of the specified vacuum in the air-flow procedure.

The relatively large methods variance for all samples except the reference sample (sample I) in Table 3 arises mainly from the condition that with these materials oven drying yielded results 0.76 to 3.03 per cent higher than either of the other methods (Table 4). These differences were to be expected and need no further discussion at this juncture, for it is known that oven drying expels more or less water of crystallization from common hydrated fertilizer salts, whereas the other methods are supposed to show free water only. Considerable interest lies in the comparison of vacuum desiccation with the air-flow method (Table 4, last column). With the exception of the reference sample, in which case the results are practi-

TABLE 4.—*Comparison of method performances on individual samples*

SAMPLE	DIFFERENCE BETWEEN METHOD MEANS		
	A-B	A-C	B-C
I. Potassium sulfate	0.01	0.01	0.00
II. Superphosphate	3.03***	2.14***	-0.89***
III. Cocoa shell meal	2.69***	1.96***	-0.73***
IV. 10-6-4 fertilizer	2.74***	2.49***	-0.25
V. 10-10-10 fertilizer	0.89***	0.76***	-0.13
VI. 5-10-5 fertilizer	1.74***	1.39***	-0.35**

** Significant, odds 99:1.

*** Significant, odds 999:1.

Significance was tested against the combined variances of within replicates and the interaction of collaborators with methods.

cally identical, vacuum drying gave lower results than the air-flow method and the differences attained statistical significance in three of the five samples.

The differences between the results by vacuum-desiccation and air-flow methods merit further discussion, since both methods are thought to give a measure of the free water in the sample. One suggestion would be that 5 hours is insufficient time for vacuum drying. That it sometimes is, whereas other times it is not, is illustrated by the drying-rate curves for superphosphate and cocoa shell meal shown in Figure 3. These materials were similar to, but not identical with, the materials used in the moisture study. In the case of the cocoa shell meal the weight loss was still increasing at a moderate rate at the end of 5 hours—an observation that affords a plausible explanation for the poor showing of vacuum drying on this type of material (Table 2). Cocoa shell meal is hardly a fair test of the method, but similar rate curves have been encountered with mixed fertilizer samples. Thus, a longer desiccation period, preferably overnight (16-18 hours), is indicated for a general procedure. If overnight desiccation had been used in the collaborative study, the differences between the re-

sults by the two methods would no doubt be lower, but hardly below the level of significance in all instances—in the case of cocoa shell meal, for example.

Finally, a few remarks should be made relative to the reproducibility of the results recorded in Table 2. If one of the collaborators, a random selection, were to repeat the air-flow determination on sample VI, for example, the probability (with the usual assumptions as to sampling and

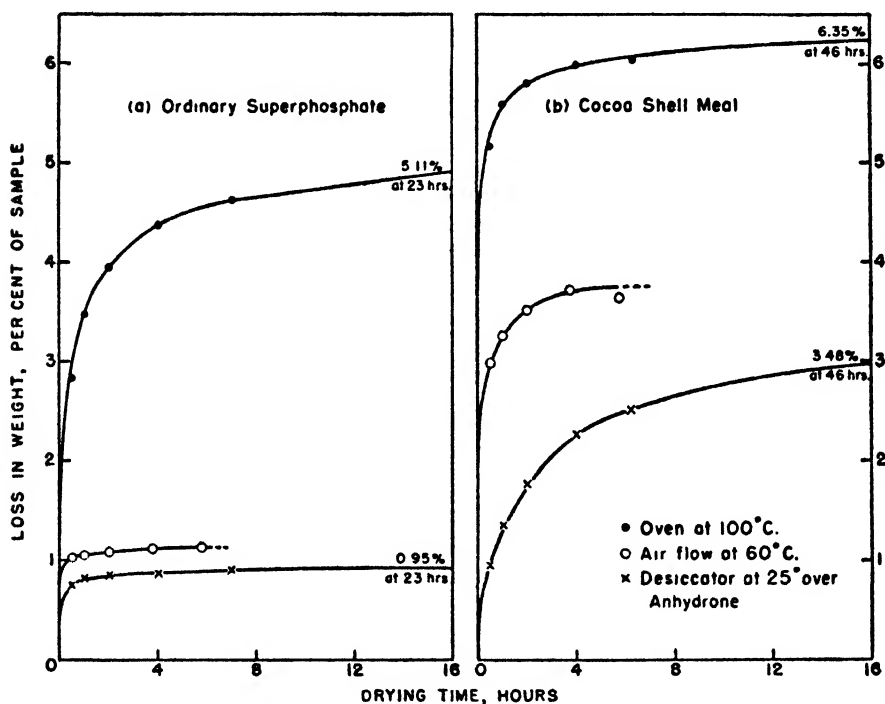


FIG. 3.—Weight loss of superphosphate and cocoa shell meal dried by different methods.

distribution) that the new result would lie within the range, recorded mean \pm standard deviation, that is to say, within 3.90 ± 0.23 , is about 2 to 1. Similarly, the odds in favor of the new result falling within 3.90 ± 0.46 are about 19 to 1. On the other hand, if all ten of the collaborators were to repeat the work on this sample, the odds in favor of the new mean differing from the recorded mean by less than ± 0.16 are 19 to 1. An interesting comparison can be drawn without recourse to probability. Thus, the difference between the extreme collaborator means for the air-flow procedure on all samples except I and III is of the order of 1 per cent or less, which would give rise to a difference of about 0.2 per cent, or less, between "moisture-free" results for P_2O_5 in a sample that contains 20 per cent of P_2O_5 .

GENERAL CONCLUSIONS

The performance of the air-flow method in the hands of the collaborators, particularly with respect to speed and to consistency of results on mixed fertilizers, would seem to justify its recommendation for use as an official method for determining free water in fertilizers. It is realized that the method, which was designed for rapid routine analysis, will hardly separate all free water from water of crystallization in all mixtures that fertilizer manufacturers may prepare, but the same could be said of any other rapid method that might be proposed.

The simplicity of equipment necessary for overnight drying in a vacuum desiccator commends this procedure with overnight drying as an alternate method for determining free water in fertilizers. The lower temperature here used would seem to give this method preference for certain types of mixtures.

The official procedure for oven drying can perhaps be modified by a judicious choice of a higher temperature so as to yield useful approximate results for the sum of the free water and the water of crystallization on samples to which it can be properly applied.

SECTION B. STATUS OF DISTILLATION METHOD FOR FERTILIZERS

The procedure for determining moisture by distillation with toluene was adopted⁵ as a tentative method in 1931 upon the recommendation of the Associate Referee on High-Analysis Fertilizers, Mr. John B. Smith, who had made a fairly extensive study of it in his search⁶ for a method that could be applied to certain troublesome materials. Further work was reported⁷ in 1932, at which time extensive work was contemplated for the next year. However, the contemplated work was not carried out,⁸ and apparently no further work has been done on the method, although in 1939 it was recommended⁹ for further study and there referred to the Associate Referee on Nitrogen.

In order to obtain some idea of the place the distillation method holds currently in fertilizer analysis, a questionnaire was sent to the collaborators last summer. The questions were: (1) Have you used this method?, (2) Do you now use it?, (3) What is your opinion as to its applicability?, and (4) Give names and addresses of those whom you know are using it. None of the twelve reporting collaborators knew anyone who now uses the method on fertilizers; one of them uses it (with benzene in place of toluene) now occasionally, but only on fish scrap—so stand the replies to questions 2 and 4. The replies to the other questions are given in Table 5. The information elicited by the questionnaire, therefore, confirms the judgment

⁵ *This Journal*, 15, 86 (1932).

⁶ *This Journal*, 14, 206 (1931); 15, 272 (1932).

⁷ *This Journal*, 16, 220 (1933).

⁸ *This Journal*, 17, 251 (1934).

⁹ *This Journal*, 23, 51 (1940).

of one of the collaborators,¹⁰ whose letter accompanying his report states, among other things, "the distillation method does not include all the water of hydration and (it may) include water of constitution under some circumstances." "A stronger criticism is that it is too long and requires special equipment." "So far as I know it has not been used, and I recommend that it be taken out of the Book of Methods." Accordingly, in line with the movement¹¹ to eliminate as far as possible tentative methods from the next edition of *Methods of Analysis*, the deletion of the distillation method is recommended solely on the basis of non-use.

TABLE 5.—*Collaborators report on use and applicability of distillation method*

COLLABORATOR NO.	HAVE YOU USED THIS METHOD?		WHAT IS YOUR OPINION AS TO ITS APPLICABILITY
	YES	NO	
1	x		Do not like it, though it yields good results
3		x	Not in position to comment
4		x	Believe it should be studied further
7	x		Gives irregular results when salt hydrates are present
9	x		Believe it would be useful in special cases
11		x	Seems impractical for many determinations per day
12		x	Requires too much undivided attention
14		x	Consumes too much time
16	x		Too cumbersome for routine work
18	x		Too complicated and requires too much apparatus
20	x		Could easily have possibilities
21		x	Not in position to comment

SECTION C. PHRASEOLOGY OF PRESENT OFFICIAL PROCEDURE

Pursuant to a request by the Committee on Classification of Methods contained in a letter from the Chairman, dated June 29, 1948, certain changes in the wording of the present official procedure for oven drying were recommended in a preliminary report furnished the Secretary August 28, 1948. These proposed changes are repeated below as items A, B, and C of Recommendation 6; item D has been added since the preliminary report was written, mainly because *water*, being the more general term, involves fewer embarrassing commitments than does *moisture*.

ACKNOWLEDGMENT

In the moisture work this year aid was promised and as far as possible contributed by the following collaborators:

D. B. Bates, Smith-Douglass Company, Inc., Norfolk 1, Va.

¹⁰ John B. Smith.

¹¹ *This Journal*, 31, 63 (1948).

- H. C. Batton, Swift and Company, 219 Wainwright Bldg., Norfolk 10, Va.
 A. T. Blackwell, Supervisor, Analytical Laboratory, The Davison Chemical Corp., Baltimore 3, Md.
 L. E. Bopst, State Chemist, College Park, Md.
 C. A. Butt and W. H. Banks, International Minerals and Chemical Corp., East Point, Ga.
 E. W. Constable, State Chemist, North Carolina Dept. of Agr., Raleigh, N. C.
 M. P. Etheredge, State Chemist, Mississippi State Chemical Laboratory, State College, Miss.
 W. R. Flach, Chief Chemist, Eastern States Farmers' Exchange, Buffalo 5, N. Y.
 V. L. Fuqua, State Chemist, Tennessee Dept. of Agr., Nashville 3, Tenn.
 W. C. Geagley, Chief Chemist, and P. O'Meara, Michigan Dept. of Agr., Lansing 13, Mich.
 Howard Hammond, Chemist, and J. S. Overholzer, State Laboratories Dept., Bismark, N. D.
 R. C. Koch, Chemist, Plant Food Div., Swift and Company, Hammond, Indiana
 Frank J. Kokoski, Chemist, New York Agr. Expt. Sta., Geneva, N. Y.
 Allen B. Lemmon, Chief, and J. B. La Clair, Bureau of Chemistry, California Dept. of Agr., Sacramento 14, Calif.
 C. T. McCloud, Chemist, F. S. Royster Guano Company, Norfolk, Va.
 P. McG. Shuey, President, Shuey and Company, Savannah, Ga.
 H. L. Moxon, Chemist, Virginia-Carolina Chemical Corp., Richmond, Va.
 John B. Smith, Chemist, and Roland Gilbert, Rhode Island Agr. Expt. Sta., Kingston, R. I.
 Marvin Van Wormer, Fertilizer Div., The Farm Bureau Cooperative Assoc., Inc., Columbus 16, Ohio
 R. D. Wallace, Technical Supt., Spencer Chemical Company, Pittsburg, Kans.

SECTION D. RECOMMENDATIONS*

It is recommended—

(1) That the air-flow method be made official for determining free water in fertilizers (first action).

(2) That the vacuum-desiccation method with a drying period of 16 to 18 hours be made official for determining free water in fertilizers (first action).

(3) That the official procedure for oven drying be modified to state only one drying temperature, the selection of the temperature to be determined by study, not necessarily collaborative, during the coming year.

(4) That further study be made on the applicability of the aforementioned three methods.

(5) That the tentative method for determining moisture with the use of distillation with toluene be deleted from the "Methods of Analysis."

(6) That the phraseology of the present official procedure for oven drying be modified as follows:

(A) Change parenthetical remark now worded "Not applicable to samples containing compounds other than H_2O that are volatilized at the temp. of drying." to read "Not applicable to samples that yield volatile substances other than H_2O at the temp. of drying."

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 43 (1949).

(b) Change first sentence of text now worded "Heat 2 g of prepared sample, 2.2, for 5 hours in water oven at temp. of boiling water (98–100°)." to read "Heat 2 g of prepared sample, 2.2, for 5 hours in oven at temp. of 99–101°."

(c) Change second sentence of text now worded "In case of potash salts, NaNO_3 , and $(\text{NH}_4)_2\text{SO}_4$, heat at ca 130° to constant weight." to read "In case of NaNO_3 , $(\text{NH}_4)_2\text{SO}_4$ and potash salts heat to constant weight in oven at temp. of 129–131°."

(d) Change section heading to read "Water" instead of "Moisture."

No report was given on boron in fertilizers.

The contributed paper "Determination of Borax in Mixed Fertilizers," by Donald S. Taylor, is published in *This Journal*, p. 422.

REPORT ON NITROGEN IN FERTILIZERS

By M. P. ETHEREDGE (Mississippi State Chemical Laboratory,
State College, Mississippi), *Associate Referee*

The report to the Association of last year (*This Journal*, 31, 236, 1948) indicated the lack of agreement on a method for total nitrogen in ammonium nitrate; therefore, it was agreed to continue studies on the analysis of this product. Owing to the possibility of the rapid formaldehyde titration procedure, it was decided to concentrate on this method and compare it to the Devarda Method.

Even more discouraging last year were the results on a high nitrate-chloride mixture. Here, again, was an opportunity for continued study, with the hope for some improvement. It was later decided to also include one sample of a low nitrate-chloride mixture.

There was a feeling among several chemists of the Association that we should revive the comparison of the Devarda Method with method 2.27 when applied to nitrate of soda. Method 2.27 is the Kjeldahl Method Modified to Include Nitrogen of Nitrates, and has the option of using either zinc or thiosulfate for reduction.

Some will remember when this comparison was made several years ago. The initial study was made by I. K. Phelps as Associate Referee, and the study was continued after A. L. Prince became Associate Referee. References to these studies are as follows: *This Journal*, 5, 450 (1922); *ibid.*, 6, 391 (1923); *ibid.*, 7, 381 (1924); *ibid.*, 8, 410 (1925). It has been said that these results were inconclusive; therefore, it was thought well to send out one sample to revive this argument.

SAMPLES

With the foregoing in mind, five samples were sent out during March to thirty-three collaborators. The descriptions of the samples follow:

(1) *Pure Ammonium Nitrate*.—This was prepared by Ralph D. Miller of Spencer Chemical Company. Owing to small impurities that yet remain, it contains approximately 34.91% Nitrogen.

(2) *Commercial Ammonium Nitrate*.—This is a regular fertilizer grade that is sold by Spencer Chemical Company. It contains approximately 34.28% Nitrogen.

(3) *High Grade Nitrate-Chloride Mixture*.—This sample was carefully prepared in the Mississippi State Chemical Laboratory from 70 per cent of reagent grade nitrate of soda, and 30 per cent superphosphate-muriate mixture. The analysis should be approximately 11.55–3.05–9.70.

(4) *Low Grade Nitrate-Chloride Mixture*.—This mixture was prepared by taking 50 per cent of last year's sample No. 3 and the other half is superphosphate with a bit of limestone added. The analysis is approximately 5.50–11.30–5.40.

(5) *C. P. Sodium Nitrate*.—This is a reagent grade product (J. T. Baker) and the nitrogen content should approach the theoretical, 16.47%.

DIRECTIONS FOR COLLABORATORS

A. Samples No. 1 and 2 are to be run by the Devarda Method (2.31). Also, they are to be run by formaldehyde titration procedure as follows:

NITROGEN IN AMMONIUM NITRATE

Formaldehyde Titration Method

(May also be adapted to Ammonium Sulfate)

Weigh out 7.004 or 14.008 g of sample and make up to 250 or 500 ml. Pull off 25 or 50 ml and put into a 300–500 ml Erlenmeyer (ca. 1.5 g may be rapidly weighed and washed directly into flask). Add ca. 1 ml of reagent formaldehyde for each 0.1 g of sample in aliquot. Make total volume 150–200 ml and allow 5 min. before titrating with 0.25–0.50 *N* sodium hydroxide, using 5 drops of phenolphthalein as indicator. Titrate until there is no perceptible color change at the point of contact, or until the proper color of pink persists. Run a blank on the formaldehyde.

$$\% \text{ Nitrogen} = \frac{\text{Net ml of NaOH} \times \text{Normality} \times 2.8016}{\text{Wt. of Sample}}$$

B. Samples No. 3 and 4 are to be run by 2.27, part 2, as amended by Mr. Ford.

We would also like these samples to be run by 2.27, part 1, using 2 gm of salicylic in 30 ml of approximately 15% fuming sulfuric (use 0.7004 gm sample in No. 3).

Mr. Shuey would also like for his procedure to be tried again, using a Millican Spiral or Filter Disc gas washing bottle instead of the Drechsel bottle.

C. Run the nitrate of soda by Mr. Ford's procedure and by the Devarda Method.

We believe that all nitrates should be dried and reported as if moisture free. Please give a moisture on samples No. 3 and 4.

We are particularly interested in adopting the formaldehyde procedure; therefore, please give us your version of the best detail of the method. We can then set up an overall compromise procedure.

RESULTS

A reasonably good response from collaborators was obtained, and the Associate Referee feels deeply grateful to the following persons, who have made this report possible.

Ralph D. Miller, Spencer Chemical Co., Pittsburg, Kansas
W. A. Morgan, Ammonia Dept., Du Pont, Wilmington

R. L. Willis & A. C. Wark, New Jersey Exp. Sta., New Brunswick
W. S. Thompson, Dept. of Agr., Columbus, Ohio
C. A. Butt & W. H. Banks, Int. Min. & Chem. Co., East Point, Ga.
C. Reynolds Clark, State Chemist, Atlanta, Ga.
R. C. Koch, Swift & Co., Hammond, Ind.
A. N. Lineweaver, F. S. Royster Guano Co., Norfolk, Va.
Geo. E. Grattan & R. Payfer, Dept. of Agr., Ottawa
G. C. Bollinger & C. M. Fleming, Am. Agr. Chem. Co., Baltimore
Howard Hammond, Chemist, State Laboratories, Bismarck, N. D.
Frances L. Bonner, Feed & Fert. Lab., Baton Rouge, La.
O. W. Ford, Indiana Exp. Sta., Lafayette
W. C. Geagley & Percy O'Meara, Bureau Chem. Labs., Lansing, Mich.
Gordon Hart, Asst. State Chem., Tallahassee, Fla.
P. R. Bidez, Senior Chemist, Auburn, Ala.
A. F. Spelman, Senior Chemist, Mass. Exp. Sta., Amherst
V. G. Hiatt, Asst. Chem., Dept. Agr., Salem, Oregon
Guy Mitchell, Chief Chemist, Lion Oil Co., Eldorado, Ark.
H. R. Allen, Chemist, Ky. Agr. Exp. Sta., Lexington
Philip McG. Shuey, Savannah, Ga.
C. O. Willits, in charge Analyt. Section, Eastern Reg. Lab., Philadelphia
Roland Gilbert, Rhode Island Exp. Sta., Kingston
C. O. Hurst, Mississippi State Chem. Lab., State College

The results are shown in Tables 1, 2, and 3. Both ammonium nitrates are reported in Table 1 for ease of comparison of the Devarda and formaldehyde procedures. The high nitrate-chloride mixture is the only sample reported in Table 2. Finally, the low nitrate-chloride mixture is reported in Table 3 with the nitrate of soda.

DISCUSSION OF RESULTS

Ammonium Nitrate.—If the results on the ammonium nitrates are examined, it is seen that the agreement in the majority of instances is very good. Some have always claimed difficulty with the Devarda Method, and it is not too easy to decide on the end point in the formaldehyde titration.

It might be well to give a detailed answer to the questionnaire regarding the formaldehyde procedure; however, a summary will probably suffice.

Seven collaborators prefer to titrate until there is no perceptible color change at the point of contact while four prefer a definite color that persists. Three suggested potentiometric ranges from pH 8 to 8.4. Analyst No. 15 probably has the best suggestion when he says allow each analyst to adjust the titration to his own wishes when compared to a reagent grade sample.

Seven collaborators prefer quick weighings of approximately 1.5 gram of sample and eight prefer making an exact amount up to volume and taking an aliquot.

There are seven collaborators who think the titration procedure is preferred while an equal number think that it should be regarded only as an

alternative procedure to the Devarda Method. Two definitely prefer the latter.

Nitrate-Chloride Mixtures.—In the case of the high nitrate-chloride mixture, few obtained the theoretical amount by the fuming sulfuric pro-

TABLE 1.—*Total nitrogen in ammonium nitrate*

ANALYST	SAMPLE NO. 1		SAMPLE NO. 2	
	DEVARDA METHOD	FORMALDEHYDE TITRATION	DEVARDA METHOD	FORMALDEHYDE TITRATION
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	34.54	34.83	34.23	34.36
2	34.99	34.93	34.18	34.28
3	34.92	34.70	34.14	33.90
4	34.98	34.10	34.21	33.70
5	34.99	34.96	34.25	34.31
6	34.89	34.86	33.99	34.08
7	34.96	34.94	34.25	34.26
8	34.87	34.76	34.40	34.24
9	34.70	34.50	34.20	33.80
10	35.05	34.90	34.40	34.29
11	34.78	34.90	34.14	34.26
12	34.52	34.33	34.33	34.20
13	35.02	34.97	34.32	34.28
14	35.05	34.69	34.44	34.02
15	34.73	34.86	34.21	34.24
16	34.97	35.04	34.30	34.36
17	34.84	34.73	34.42	34.08
18	34.65	34.59	34.05	34.07
19	—	34.89	—	34.26
20	34.96	35.15	34.28	34.38
21	34.97	34.97	34.45	34.48
22	35.01	34.87	34.40	34.14
23	34.56	35.02	33.91	34.32
24	34.72	34.90	34.10	34.10

cedure. None approached the theory by the Shuey Method, although the results as a whole were not extremely low.

The Ford version of the Kjeldahl Method to Include Nitrates (2.27, part 2) seems promising. Over a third of the analysts obtained or approached the theoretical amount. On the other hand, there were some very low results.

The results by the Ford version on the low nitrate-chloride mixture are even better than on the high mixture. This, of course, would be expected. There are also a few good results by the other two procedures. A few high results are indicative that this sample may not have been properly mixed.

Nitrate of Soda.—There were three analysts who obtained as high or

slightly higher results on the nitrate of soda by the Ford procedure. However, most analysts obtained slightly higher results by the Devarda

TABLE 2.—*Sample No. 3. Total nitrogen in high grade nitrate-chloride mixture*

ANALYST	FORD METHOD	SHUEY METHOD	FUMING SULFURIC	MOISTURE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	10.88	10.35	7.92	0.40
2	11.57	11.26	5.56	0.34
3	11.53	11.05	11.45	0.68
4	10.76	—	—	0.44
5	11.47	11.29	4.56	0.56
6	11.58	—	11.61	0.67
7	11.23	11.23	11.00	1.53
8	9.85	—	9.68	0.60
9	11.40	10.40	9.80	1.01
10	11.39	11.20	9.49	0.60
11	8.18	10.25	—	0.46
12*	11.24	11.12	11.42	—
13	11.51	—	—	0.52
14	11.46	—	11.30	0.61
15	11.20	11.16	11.34	0.64
16	11.30	—	7.18	1.22
17	11.07	—	—	0.69
18	10.98	—	—	—
19	—	—	—	—
20	11.37	10.32	10.76	0.67
21	11.13	11.29	—	—
22	11.52	—	11.68	0.40
23	11.30	—	—	0.71
24	11.48	—	—	0.49

* Dry basis

Method. If one will refer back to the previous references on this subject he will find that this was somewhat the case twenty-five years ago.

CONCLUSIONS

(1) It is obvious that the Devarda Method and the formaldehyde titration procedure give about equal results on the analysis of ammonium nitrate for total nitrogen. Most of the analysts prefer the latter's adoption as either a preferential or alternative method. The end point is not ideal; however, it can be adapted to any laboratory. Most laboratory analysts who have only a few ammonium nitrates would probably prefer to use the Devarda Method. Most analysts will agree that the formaldehyde procedure can be used to good advantage where there are large numbers of samples.

(2) The high nitrate-chloride mixtures will not be easy to solve. It may

TABLE 3.—*Total nitrogen*

ANALYST	SAMPLE NO. 4 (LOW NITRATE-CHLORIDE)			SAMPLE NO. 5 (NITRATE OF SODA)	
	FORD METHOD	SHURT METHOD	FUMING SULFURIC	DEVARDA	FORD METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	5.21	4.99	4.69	16.34	16.27
2	5.73	5.69	3.90	16.43	16.21
3	5.55	5.28	5.62	16.41	16.31
4	5.25	—	—	16.44	—
5	5.76	5.63	3.67	16.42	16.36
6	5.48	—	5.65	16.46	16.01
7	5.37	—	5.57	16.22	16.23
8	5.24	—	5.30	16.25	15.90
9	5.60	5.00	4.80	16.40	16.00
10	5.70	5.65	5.65	16.46	16.45
11	5.50	5.68	—	16.36	16.17
12	5.67	5.48	5.68	16.40	16.38
13	5.60	—	—	16.45	16.47
14	5.62	—	5.66	16.19	16.16
15	5.52	5.50	5.63	16.34	16.31
16	5.51	—	4.45	16.40	16.35
17	5.56	—	—	—	16.38
18	5.55	—	—	16.37	16.42
19	—	—	—	—	—
20	5.73	5.14	5.46	16.40	—
21	5.48	—	—	16.48	16.39
22	5.84	—	5.89	16.44	15.96
23	5.59	—	—	16.20	16.29
24	5.62	—	—	16.36	16.33

be that some variation of 2.27 as suggested by O. W. Ford will be the most practical method. Perhaps there is a limit beyond which no practical method will give good results.

(3) There is no occasion for extreme arguments in the case of nitrate of soda. A few samples can be run with a reasonable degree of accuracy by 2.27. However, analysts who have had the nitrate of sodas by the hundreds will prefer the Devarda Method. The checks may be wider by the latter; however, results will more nearly approach the theoretical amount of nitrogen.

RECOMMENDATIONS*

It is recommended—

(1) That the formaldehyde titration method be adopted as official, first action, for determining nitrogen in ammonium nitrate.

(2) That the study of nitrogen in high nitrate-chloride mixtures be continued.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 43 (1949).

REPORT ON POTASH

By O. W. FORD (Purdue University Agricultural Experiment Station,
West Lafayette, Indiana), *Associate Referee*

In accordance with the recommendations of the Association "that work on the details of the method for the determination of potash in fertilizers be continued" (*This Journal*, 31, 42, 1948) referee work was conducted in 1948 by collaboration.

A copy of the proposed work was sent to each chemist who had expressed a willingness to collaborate. This report summarizes the results of the twelve chemists who found time to do the work and report to the Associate Referee.

Collaborative work was directed to a comparison of potash and insoluble phosphoric results obtained on fertilizers when like portions of the same sample were ground in the old type Burr mill and the new type Micro-samplmill. The new type Micro-samplmill is a hammer mill in which the hammers are fixed on a rotor. This mill was one of two mills exhibited and demonstrated at the 1947 A.O.A.C. Meeting.

This work was undertaken because early trials in the Indiana Laboratory on some late 1947 fall shipments of fertilizer indicated that slightly lower insoluble phosphoric acid and higher potash values might be obtained by its use.

The lower insoluble phosphoric acid values agreed with work previously reported by Ross and Jacobs, etc., when samples were more finely ground.

1948 COLLABORATIVE WORK ON POTASH IN FERTILIZERS

With the recent use of the Micro-samplmill an increase in both potash and available phosphoric acid values have been obtained in the Indiana Laboratory over that obtained with portions of the same sample ground in a Burr mill. If this is universally true, it would be worthy of some collaborative work.

Six samples have been ground for analysis in each of the two types of mills.

Type 1 <i>Burr Mill</i>		Type 2 <i>Micro-samplmill</i>	
3841	8-10-10	3841	8-10-10
3720	4-12- 8	3720	4-12- 8
3683	3- 9-18	3683	3- 9-18
3684	3-12-12	3684	3-12-12
4069	0- 9-27	4069	0- 9-27
3999	3-12-12	3999	3-12-12

It is recommended:

1. Burr Mill Samples—

Make three individual potash determinations on each sample.

Make three individual insoluble phosphoric acid determinations on each sample.

2. Micro-samplmill Samples—

Make three individual potash determinations on each sample.

Make three individual insoluble phosphoric acid determinations on each sample.

It should be understood that the present official method for potash will be used, and that all collaborators will use 95% ethyl alcohol or 95% formula 30 alcohol and the corresponding acid-alcohol for the potash determinations.

Dr. K. D. Jacob, Associate Referee on phosphoric acid, has sanctioned the work on the insoluble phosphoric acid.

COMMENTS ON RESULTS

The results of the twelve chemists who collaborated on the potash and insoluble phosphoric acid are reported in Tables 1 and 2.

Taken as a whole, the potash results are slightly more disappointing than the Associate Referee had expected. Only two of the six samples showed a slightly higher average of potash values when ground by the Micro-samplmill over that of the Burr mill.

This, however, is in agreement with one of the collaborators, who reported that considerably less potash was obtained in his laboratory when samples were ground too fine. It seems difficult to believe that fine grinding would produce less potash. This is also a little contradictory to past work on potash, where slightly more potash and more concordant results were obtained. One collaborator offered the suggestion that perhaps some segregation has taken place. If this is the case, there is a definite need for a thorough study of the effect of fineness of grinding and an investigation of the proper procedure of drawing a sample for analysis when a sample has been prepared for analysis. This should be taken into consideration since there is no definite instruction regarding this at present. Reports to the Associate Referee indicate that all but one of the manufacturing chemists remixed on paper all samples sent to them for analysis while the control chemists followed a procedure of drawing the sample directly from the bottle. It is the observation of the Associate Referee that many samples that have been thoroughly mixed can be segregated by remixing on paper. A survey of the results obtained indicate that the greatest variations from the average occurred when the collaborator removed the sample from the bottle and remixed it. Many but not all collaborative chemists concur in this procedure. The Associate Referee regrets the fact that definite instruction was not given to the collaborators to draw all samples directly from the bottle. In nearly all cases all collaborators obtained slightly less insoluble phosphoric acid on the Micro-samplmill samples. These differ-

TABLE 1.—*Effect of fineness of grinding on the determination of potash in fertilizers*

ANALYST NUMBER	SAMPLING PROCEDURE	BUER MILL					MICRO-SAMPLING						
		AVERAGE OF THREE ANALYSES PER CENT POTASH					AVERAGE OF THREE ANALYSES PER CENT POTASH						
		SAMPLES					SAMPLES						
		3683	3684	3720	3841	3999	4069	3683	3684	3720	3841	3999	4069
1	*	16.72	12.20	7.89	10.13	11.19	26.58	16.24	12.03	7.85	9.95	11.29	26.68
1	†	16.72	12.12	7.99	10.10	11.18	26.97	16.21	12.03	7.82	9.92	11.32	26.79
2	*	16.63	12.37	7.83	10.25	11.08	26.67	16.10	11.94	7.63	9.84	11.10	26.58
3	*	17.23	12.65	8.39	10.46	11.32	27.20	16.91	12.30	8.26	10.18	11.63	27.31
4	*	16.64	11.92	7.85	10.16	11.24	26.73	16.38	11.72	7.65	9.99	11.27	26.78
5	†	17.07	12.64	7.94	10.62	11.32	28.21	17.13	12.18	7.90	10.25	11.27	28.66
6	†	16.64	12.26	7.82	10.21	11.02	26.65	16.24	11.89	7.72	9.90	11.15	26.54
8	*	16.92	12.45	7.88	10.20	11.34	27.47	16.41	12.01	7.72	10.04	11.40	27.22
9	†	17.09	12.48	7.96	10.46	11.20	27.08	16.79	12.02	7.81	10.04	11.37	27.19
10	*	17.00	12.75	8.02	10.48	11.58	27.48	16.60	12.45	8.02	10.33	11.88	27.41
12	*	16.80	12.34	8.00	10.09	11.07	27.21	16.39	12.22	7.83	10.13	11.33	26.96
13	*	16.25	12.08	7.56	9.98	11.28	25.80	16.10	12.05	7.60	9.95	11.32	25.86
15	†	16.98	12.45	8.20	10.49	11.72	27.57	16.60	12.52	8.20	10.23	11.82	27.65
High		17.23	12.75	8.39	10.62	11.72	28.21	17.13	12.52	8.26	10.33	11.88	28.66
Low		16.25	11.92	7.56	9.98	11.02	25.80	16.10	11.72	7.60	9.84	11.10	25.86
Average		16.83	12.40	7.93	10.31	11.30	27.03	16.50	12.11	7.85	10.06	11.42	27.09
Maximum variation		0.98	0.67	0.83	0.64	0.70	2.41	1.03	0.63	0.66	0.49	0.78	2.80

* Sample weighed from bottle, mixed on paper and several dips taken for each sample.

† Sample weighed directly from bottle.

TABLE 2.—Effect of fineness of grinding on the determination of insoluble phosphoric acid in fertilizers

ANALYST NUMBER	SAMPLING PROCEDURE	BUER MILL						MICRO-SANTAMILL					
		AVERAGE OF THREE ANALYSES PER CENT INSOLUBLE P ₂ O ₅						AVERAGE OF THREE ANALYSES PER CENT INSOLUBLE P ₂ O ₅					
		SAMPLES						SAMPLES					
		3683	3684	3720	3841	3999	4069	3683	3684	3720	3841	3999	4069
1	*	0.35	0.41	0.70	0.47	1.22	0.39	0.34	0.35	0.63	0.48	1.15	0.30
1	†	0.32	0.41	0.68	0.44	1.18	0.38	0.37	0.38	0.62	0.50	1.17	0.37
2	*	0.35	0.30	0.71	0.41	1.34	0.26	0.29	0.28	0.67	0.44	1.22	0.25
3	*	0.45	0.55	0.94	0.60	1.42	0.52	0.55	0.43	0.95	0.77	1.34	0.53
4	*	0.40	0.43	0.80	0.51	1.42	0.32	0.37	0.33	0.72	0.45	1.38	0.32
5	†	0.35	0.34	0.76	0.46	1.21	0.29	0.32	0.36	0.72	0.54	1.31	0.31
6	†	0.39	0.40	0.88	0.44	1.29	0.33	0.34	0.29	0.73	0.50	1.22	0.30
8	*	0.34	0.42	0.83	0.47	1.34	0.34	0.33	0.36	0.77	0.48	1.30	0.32
9	†	0.38	0.33	0.88	0.53	1.12	0.40	0.37	0.30	0.77	0.45	1.08	0.42
10	*	0.35	0.35	0.92	0.42	1.28	0.25	0.36	0.35	0.72	0.43	1.33	0.27
12	*	0.32	0.38	0.78	0.44	1.16	0.30	0.36	0.36	0.76	0.46	1.22	0.32
13	*	0.38	0.40	0.68	0.53	1.18	0.36	0.36	0.35	0.66	0.50	1.13	0.26
15	*	0.34	0.38	0.84	0.44	1.06	0.25	0.27	0.24	0.75	0.44	0.92	0.22
High		0.45	0.55	0.94	0.60	1.34	0.52	0.55	0.43	0.95	0.77	1.33	0.53
Low		0.34	0.30	0.68	0.41	1.06	0.25	0.27	0.24	0.63	0.43	0.92	0.22
Average		0.37	0.39	0.81	0.48	1.27	0.34	0.35	0.33	0.74	0.50	1.21	0.32
Maximum variation		0.13	0.25	0.26	0.19	0.28	0.27	0.28	0.19	0.32	0.34	0.41	0.31

* Sample weighed from bottle, mixed on paper and several dips taken for each sample.

† Sample weighed directly from bottle.

ences were not as great as might have been obtained had samples been selected with larger insoluble phosphoric acid values.

In general, a quicker job of grinding is done by the Micro-samplmill. This will remove the danger of moisture changes during the process of preparation of the sample. Since the grinding requires less than one minute to grind a one pound sample, the tendency, due to heating, to change the composition of the sample is reduced to a minimum. With the use of the one-eighth inch screen, no samples were encountered in the Indiana Laboratory in the spring of 1948 that could not be ground in less than a minute. This is less than one-tenth of the time formerly required to grind the same amount of sample. The cleaning of the mill between samples is no greater per sample than with the Burr type mill. For this reason, the Associate Referee will recommend that these studies be continued to take in other types of mills and a greater variety of fertilizer mixtures.

LIST OF COLLABORATORS

1. W. R. Austin and Madalane Buford, Armour Fertilizer Works, Nashville, Tenn.
2. H. C. Batton, Swift & Company, 219 Wainwright Building, Norfolk, Va.
3. R. D. Caldwell, Armour Fertilizer Works, 350 Hurt Building, P. O. Box 1685, Atlanta 1, Ga.
4. William Chapman, Consolidated Rendering Company, 178 Atlantic Avenue, Boston 10, Mass.
5. T. L. Ogier and J. F. Fudge, Texas Agricultural Experiment Station, College Station, Tex.
6. R. C. Koch, Swift & Company, 150 Marble St., Hammond, Ind.
7. John W. Kuzmeski, Massachusetts State College, Amherst, Mass.
8. H. L. Moxon, Virginia-Carolina Chemical Corporation, Richmond, Va.
9. Richard M. Smith, Agricultural Department, Chemical Division, Tallahassee, Fla.
10. A. N. Lineweaver and S. F. Thornton, F. S. Royster Guano Company, Norfolk, Va.
11. L. S. Walker, University of Vermont, Burlington, Vt.
12. A. T. Blackwell, The Davison Chemical Corporation, Baltimore, Md.
13. C. W. Byers, Armour Fertilizer Works, Carteret, N. J.
14. Wm. McAllister, Director of Laboratories, Southern States Laboratories, 2101 East Fort Ave., Baltimore 30, Md.
15. R. F. Serro and O. W. Ford, Purdue University, Department of Agricultural Chemistry.

COMMENTS OF COLLABORATORS

(1) "First set of results obtained by mixing sample on paper and dipping. This is common practice on all our work at Nashville. The second set of results were obtained by weighing directly from the bottle as you do in Lafayette. Some of the samples were set up hard when received. The matter of size of sampling rod in use in the field for certain types of materials in the fertilizer mixtures needs to be investigated."

(2) "The samples, particularly the micro-mill grindings, were so tightly packed in the bottles that we thought it best to withdraw the entire portion from each bottle, break up the solidified portions, mix and re-bottle before using."

(4) "In most all cases the insoluble phosphoric acid was lower on the finer

ground sample but in only one case was the potash higher. Checking back I found in four cases there was enough total phosphoric acid to make the potash difference. In the other case a nitrogen overage will account for some of the difference. I think that in cutting down to such small samples segregation has reared its ugly head and caused the results to come out the way they did. The results on the lower insoluble phosphoric figures check out what we have found here in working on high analysis goods."

"I weighed the samples for analysis after I had removed them from the small bottle and thoroughly mixed them. Then I placed the sample in a larger bottle so that in withdrawing the sample for analysis I could keep the sample well mixed at all times. This was borne out by several checks that I made weighing out a new portion each time."

"The micro-samplmill would not operate successfully under our conditions. Samples high in water gummed up around the rotor. Suggested modification by the manufacturer was not feasible as it would have altered the sample."

(6) "Since our potash figures did not agree with work done in your laboratory, we repeated the potash determinations with substantially the same results."

(8) "As to sample grinding, this laboratory has been collaborating with a company manufacturing a machine similar to the type of machine which you have. Exhaustive tests have been made on various fertilizers and fertilizer materials. The grinding for laboratory weighings is excellent, but we have found that the solubility, especially on nitrogen, will change. This change is not uniform; some samples will increase materially while others stay fairly constant and for this reason we are unable to grind very many samples in our regular work."

"I hope the A.O.A.C. will make an investigation of machines of this type and if found satisfactory will adopt methods for their use."

(9) "Our results would appear to indicate that the Micro-samplmill grinds samples too fine for full leaching of potash in routine work by the A.O.A.C. method."

"These samples were run under routine conditions in which there is not time to stop and remix on paper every bottle of ground sample sent to the chemist. The worth of any mill to the commercial laboratories depends a lot on small items such as this."

(10) "A study such as the one proposed by you is very necessary now that it seems we are going into the use of sample grinding mills."

(12) "Samples were spread and mixed before portions were taken for analysis."

(13) "Samples spread on paper and mixed before portions were taken for analysis."

(15) "Samples drawn directly from bottles as all laboratory samples are so prepared for analysis. Results on insoluble phosphoric acid were in accord with original work, but in only three of the six cases was the potash higher on the Micro-samplmill samples. It would appear that more care in mixing the sample for analysis is necessary when samples are ground in the Micro-samplmill."

RECOMMENDATIONS*

It is recommended—

(1) That a survey be made of the different types of mills being used for preparation of the sample.

(2) That collaborative potash work on samples prepared by the different mills be conducted on a greater variety of samples.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 43 (1949).

ACKNOWLEDGMENT

The thanks of the Associate Referee are extended to F. W. Quackenbush of the Agricultural Chemistry Department, Purdue University, for valuable suggestions and criticisms in the development of this report. In addition, thanks are extended to the other collaborators for their cooperation.

No reports were given on magnesium and manganese, acid- and base-forming quality, sulfur, copper and zinc, boron, or inert materials.

A paper entitled "Limitations of the Modified Kjeldahl Method for Determining the Nitrate Nitrogen in Nitrate-Chloride Mixtures," by H. K. White and O. W. Ford, is published in *This Journal*, p. 397.

REPORT ON CEREAL FOODS

By V. E. MUNSEY, (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

In anticipation of the publication of the Seventh Edition of the "Methods of Analysis" in 1950, the Referee has reviewed the Cereal Food Chapter No. 20, looking toward either final adoption or deletion of methods. There are 7 methods for various proteins (20.32-38, incl.) which were developed many years ago and have appeared as tentative methods in various editions of the "Book of Methods." These methods do not determine a specific component of flour. They are antiquated and little used except in some research work. It is recommended that these methods be dropped. There have been considerable advances in the past ten years in technical methods for the study of protein systems, which should be applied to research on cereal proteins.

The tentative method for the detection of rye flour in wheat flour, 20.60 is of doubtful value, and it is recommended it be dropped. Further consideration will be given other methods that are not official during the coming year.

RECOMMENDATIONS*

The following recommendations are based on the work of the Associate Referees:

It is recommended—

(1) That both procedures proposed by the Associate Referee last year (*This Journal*, 31, 79) for the determination of phosphorus in cereals and cereal products be adopted as official (final action) and the study be discontinued.

(2) That the studies on determination of starch in raw and cooked cereals be continued.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

(3) That the tentative method for the determination of fat acidity in grain and flour (20.18–20.21, incl. and 20.76) be adopted as official (first action) and the study continued.

(4) That a study of the application of the method for reducing and non-reducing sugar in flour, 20.28–30, incl., be made to the determination of sugars in bread and other bakery products, with special consideration given to the article on this subject published by R. M. Sandstedt and G. C. Fleming (*This Journal*, 30, 550–2).

(5) That the tentative method for benzoic acid in flour (20.53) be replaced by the method proposed by the Associate Referee for wheat flour and be adopted as official (first action) as a qualitative test, and the study continued.

(6) That work on methods for determination of available CO_2 in self-rising flour, containing added CaCO_3 , be discontinued.

(7) That the methods for the determination of lactose in bread be further studied.

(8) That the method for determination of fat and fat number in bread as proposed by the Associate Referee in this year's report, replace 20.86 and be adopted as official (first action).

(9) That the method proposed by the Associate Referee in this year's report for the determination of proteolytic activity of flour and malted wheat flour be adopted as official (first action), and that the work be continued.

(10) That the methods for soybean flour—(a) moisture, 20.77 (20.4) with the exception that a 5 g sample be dried 130° for 2 hours; (b) ash, 20.78 (27.9); (c) nitrogen, 20.79, proceed as directed under 2.26, using 10 g K_2SO_4 or Na_2SO_4 , and 0.7 g HgO or its equivalent in Hg, with the additional option of using sodium alizarin sulfonate; (d) oil, 20.82, except that ca 2 g full fat flour or 5 g low or defatted soy flour be extracted for 5 hours—be adopted as official, first action, and that the study be continued.

(11) That the study on the detection and determination of soybean flour in cereal products be discontinued.

(12) That the method proposed by the Associate Referee in this year's report for the determination of added inorganic material in phosphated flour be adopted as official, first action, and the study continued with elimination of self-rising flour.

(13) That the method referred to in *This Journal*, 25, 83–84, for the determination of unsaponifiable matter and sterols in noodles, be studied to determine their applicability to bakery products containing eggs.

(14) That the study of methods for the determination of albumen in noodles and macaroni products be conducted.

(15) That the study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(16) That the study on the determination of moisture in flour products containing sodium bicarbonate as one of its constituents be continued.

(17) That the study on the determination of bromates in flour be continued.

(18) That the tentative methods 20.32–20.38, inclusive, and 20.60, be dropped.

REPORT ON THE DETERMINATION OF BENZOIC ACID IN FLOUR

By V. E. MUNSEY (Food and Drug Administration, Federal
Security Agency, Washington 25, D. C.), *Referee*

The method for the determination of benzoic acid submitted to collaborative study last year was modified on the basis of recommendations of the collaborators. The changes are largely a clarification of the directions.*

Ten collaborators were asked to analyze three samples of flour by the following procedure. The three samples consisted of an untreated flour, flour treated with usual amount of benzoyl peroxide, and a flour with about $\frac{1}{2}$ normal application of benzoyl peroxide. In the preparation of the treated flours, 825 mg of the commercial preparation containing benzoyl peroxide was mixed with 300 g of flour by sifting through a flour sifter 12 times and then mixing and rolling on paper. 100 g of this master mix was mixed in a McCellan mixer with 2500 g flour, then mixed in ball mill and rolled and mixed on paper. This final mixture represented sample No. 3, containing 21.4 p.p.m. of benzoic acid based on Associate Referee's analysis of the commercial benzoyl peroxide product.

Sample No. 2 consisted of mixing equal parts of sample No. 3 and untreated flour. It should contain 10.7 p.p.m. benzoic acid. Although it is difficult to uniformly distribute these small amounts of benzoyl peroxide in flour, the above procedure seemed to accomplish a uniform mixture.

Results received from nine chemists are given in Table 1. The results, with the exception of collaborators 6, 7, and 9, indicate a fairly close agreement in found and added amounts. One collaborator pointed out the need for absolute dryness in the test tube before nitration. This essential point may explain the low results of two collaborators. One collaborator recommended the use of 30×200 mm test tube in place of 25×150 mm as an improvement. Another collaborator suggested the use of 200 ml centrifuge bottle and decantation of the ether in preference to filtration through a Büchner funnel.

Acknowledgment is made of the splendid cooperation of the following collaborators: C. G. Harrel, Pillsbury Mills, Inc.; R. C. Koehn, General Mills, Inc.; E. Stegemeyer, Kroger Food Foundation; W. L. Rainey, Commander Larabee Milling Co.; R. L. Gray, Novadel-Agene Corp.; and F. J. McNall, M. A. Braun, and E. F. Steagall, of the Food and Drug Administration.

* Details of the method are published in *This Journal*, 32, 84 (1949).

TABLE 1.—*Benzoic acid in flour samples containing no benzoic acid, 10.7 p.p.m. and 21.4 p.p.m.*

COLLABORATOR	NO BENZOIC ACID	10.7 p.p.m.	21.4 p.p.m.
	SAMPLE 1	SAMPLE 2	SAMPLE 3
	p.p.m.	p.p.m.	p.p.m.
1	3.0	11.0	14.2
	3.2	11.0	14.6
	3.0	12.0	19.6
	av. 3.0	11.3	16.1
2	none	8.8	15.4
3	1.1	10.8	18.1
	0.8	11.9	18.8
	av. 0.9	11.3	18.5
4	1.0	9.8	19.8
	0.8	10.0	—
	av. 0.9	9.9	19.8
5	0	8.8	16.8
	—	—	14.4
			av. 15.6
6	0	4.0	6.5
7	1.5	4.0	6.0
	1.7	4.3	6.3
	av. 1.6	4.2	6.2
8	0.8	10.0	18.8
	0.9	10.6	19.8
		9.8	18.4
			20.0
	av. 0.9	10.1	19.2
9	2.3	6.3	12.1
	1.9	4.5	12.9
	2.4	7.0	12.4
	av. 2.2	5.9	12.4

It is recommended† that the method be adopted as official, first action, as a qualitative test for benzoic acid, and that study be continued on the method for improvement of the quantitative measurement.

† For report of Subcommittee D, and action of the Association, see *This Journal*, 32, 61 (1949).

REPORT ON ADDED INORGANIC MATERIAL
IN PHOSPHATED FLOUR

By FRANK H. COLLINS (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

In accordance with the recommendations of the Association (*This Journal*, 31, 59, 1948) "That the method proposed by the Associate Referee for determination of the amount of added inorganic material in phosphated flour be further studied," work has been continued this year by collaboration.

In this Associate Referee's former report (*This Journal*, 31, 259, 1948) a few collaborative results were given on flour containing added monocalcium phosphate. A modification of the carbon tetrachloride separation method of Gustafson (*This Journal*, 19, 82, 1932) was used, collecting the sediment from the separator on weighed filter paper. This material was air dried at room temperature and weighed.

In the present instance the work has been expedited by the use of a Gooch crucible. It has been found that the carbon tetrachloride is completely removed and the weight of the Gooch becomes constant by aspirating 2 or 3 minutes. The results thus obtained by direct weighing were checked, for possible error due to any flour in the sediment, by heating the crucible to 700 degrees C. and weighing as calcium metaphosphate. (Hill et al., *Ind. Eng. Chem.*, 39, 1667, 1947).

Commercial monocalcium phosphate monohydrate as used in the commercial phosphating of flour was used in the preparation of the samples. In the following tables Procedure No. I refers to the direct weighing of the sediment, while Procedure No. II refers to results obtained by heating the monocalcium phosphate monohydrate to convert it to anhydrous calcium metaphosphate. The details of the method are given in *This Journal*, 32, 88 (1949).

DISCUSSION

The sediment can generally be drawn from a separator, with a minimum of solution, by flicking or quickly turning the stop-cock from side to side. Occasionally, however, a separator will be found to have a ledge just above the stop-cock from which it is difficult to dislodge the sediment. A wire has been utilized to dislodge the deposit in such cases. The low results included in the above tables may be due to some unnoticed sediment on such a ledge.

Nearly all of the inorganic material will be in the first draw-off from the 250 ml separator. It has been found that if this is drawn off into a second separator containing carbon tetrachloride, a clean separation is made as the inorganic material settles down through the solution. It is advantageous to draw off this sediment into a weighed Gooch crucible before the

TABLE 1.—*Recovery of monocalcium phosphate added to soft red winter wheat, long patent flour*

ANALYST	PROCEDURE	SAMPLE NO. 1 CONTAINING 0.21% MONOCALCIUM PHOSPHATE	SAMPLE NO. 2 CONTAINING 0.72% MONOCALCIUM PHOSPHATE
		per cent	per cent
1	I	.21, .21, .21	.72, .73, .72
	II	.20, .20, .19	.71, .71, .71
2	I	.21, .21, .21	.72, .72, .73
	II	.20, .19, .20	.71, .71, .71
3	I	.21, .21, .21	.72, .72, .73
	II	.19, .20, .20	.71, .71, .72
4	I	.20, .20, .20	.66, .66, .66
	II	.19, .20, .19	.62, .63, .62
5	I	.20, .21, .21	.70, .47, .62
	II	.18, .19, .20	.65, .39, .60
6	I	.23, .23	.66, .68
	II	.21, .22	.63, .68
7	I	.22, .22	.70, .60
	II	.20, .20	.70, .64
8	I	.23, .23	.73, .74

TABLE 2.—*Recovery of monocalcium phosphate added to hard wheat flour*

ANALYST	PROCEDURE	SAMPLE A HARD SPRING WHEAT FLOUR CONTAINING 0.50% MONOCALCIUM PHOSPHATE	SAMPLE B HARD WINTER WHEAT FLOUR CONTAINING 0.50% MONOCALCIUM PHOSPHATE
		per cent	per cent
1	I	.50, .51, .50	.51, .50, .51
	II	.49, .50, .49	.49, .48, .49
9	I	.52, .52, .53	.50, .53, .48
	II	.49, .49, .50	.50, .50, .43

second draw-off from the 250 ml separator. Any sediment adhering to the sides of the separator may be dislodged by gently swirling the funnel and its contents. The results obtained by ignition were found to be slightly lower than those obtained by direct weighing. This is probably due in most cases to free moisture in the monocalcium phosphate monohydrate rather than to flour in the material ignited. If desired, the used carbon

tetrachloride may be re-used several times by filtering before it is necessary to distill.

The assistance of the following collaborators is gratefully acknowledged.

R. A. Barackman and D. L. Gilkey, Victor Chemical Works, Chicago Heights, Ill.

H. K. Parker, Wallace & Tiernan Co., Inc., Newark, N. J.

E. L. Sexton, The Best Foods, Inc., New York City.

E. F. Steagall, Juanita Breit, L. G. Ensminger and O. S. Keener—all of the Food and Drug Administration.

It is recommended* that this method be adopted as official, first action.

REPORT ON MILK SOLIDS AND BUTTERFAT IN BREAD

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

The tentative method, 20.86, for determination of the amount of fat and the fat number of the extracted fat for the estimation of the amount of butterfat in bread has been modified, based on further experience with the method and previous collaborative study. A 160 g sample of air dry bread and 10 g of fat was submitted to 8 collaborators with the request that fat and fat number on the bread be determined in duplicate, and the fat number on the sample of fat in triplicate. The details of the procedure are given in *This Journal*, 32, 85 (1949).

Results from six chemists were obtained, which appear in the following table. The results for fat are very good and the fat numbers are considered satisfactory for this type of determination. A value of 1.5 for maximum variation from average for fat number seems somewhat large, but in actual application it represents only 0.2 per cent butterfat in bread. This bread was made by adding a definite amount of a mixture of 44.5 per cent lard and 55.5 per cent butterfat, and contained on the air dry bread 2.2 per cent butterfat. On the basis of the Associate Referee analysis of the lard and butterfat for fat number, the determined amount of butterfat was found to be 2.4 per cent butterfat. The fat sample consisted of 54 per cent lard and 46 per cent butterfat. This fat mixture was chosen to closely approximate the butterfat content extracted from the bread due to the change caused by the flour fat. The average value of fat numbers reported by the collaborators are essentially the same for the fat extracted from the bread and the sample of fat. These results confirm previous results that the composition of the fat is not changed during extraction and that the agreement in results is similar whether determined on a uniform sample of fat or extracted fat from bread.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 62 (1949).

TABLE 1.—*Fat and fat number on air dry bread containing 2.2% butterfat, and fat number on lard-butterfat mixture*

COLLABORATOR	FAT IN AIR DRY BREAD	FAT NUMBER ON FAT EXTRACTED FROM AIR DRY BREAD	FAT NUMBER ON FAT SAMPLE
1	<i>per cent</i>		
	5.53	14.6	14.7
	5.53	14.6	15.0
	5.52	14.4	15.0
	av. 5.5	av. 14.5	av. 14.9
2	5.38	14.6	15.6
	5.37	14.6	15.5
		14.9	15.5
	av. 5.4	av. 14.7	av. 15.5
3	5.5	15.6	16.7
	5.5	15.0	16.9
	5.4	16.3	16.5
	av. 5.4	av. 15.6	av. 16.7
4	5.76	16.6	16.2
	5.65	17.0	15.6
		17.4	15.2
	av. 5.7	av. 17.0	av. 15.7
5	5.30	16.0	15.8
	5.36	16.1	15.9
		16.0	
	av. 5.3	av. 16.0	av. 15.9
6	5.4	14.8	14.7
	5.4	14.9	14.6
		15.0	
		15.0	
	av. 5.4	av. 14.9	av. 14.7
	max. 5.7	max. 17.0	max. 16.7
	min. 5.3	min. 14.5	min. 14.7
	av. 5.5	av. 15.5	av. 15.6

Further work was done on the application of the chromatographic separation of fatty acids on silicic acid column outlined in a report last year. The only variation was the substitution of 0.5 per cent *N* butyl alcohol in chloroform for 1.0 per cent in an attempt to improve the performance of the butyric acid band on the column. The fat sample and pure butterfat were analyzed. The butterfat gave 63.5, 64.8, 44.0 and 48.5 mg/g butyric acid, and the fat sample gave 29.6, 23.1, 27.6, and 18.0 mg/g. Assuming the precision may be improved, it is doubtful whether its application has

enough merit to warrant so much more time in comparison with the fat number. No collaborative work was done on the determination of lactose in bread.

The cooperation of the following collaborators is very much appreciated: E. K. Spotts, Ward Baking Co.; R. T. Bohn, General Baking Co.; and S. Kahan, Harold F. O'Keefe, and E. F. Steagall, all of the Food and Drug Administration.

It is recommended* (1) that the determination of fat and fat number in bread be adopted as official, first action, and (2) that the study on the determination of lactose in bread be continued.

REPORT ON PROTEOLYTIC ACTIVITY OF FLOUR AND OTHER PROTEOLYTIC ENZYME-CONTAINING MATERIALS†

By BYRON S. MILLER (Associate Chemist, Hard Wheat Quality
Laboratory, Manhattan, Kansas), *Associate Referee*

The Ayre and Anderson method(1) for the determination of proteolytic activity and a modification thereof (Landis, 2) have been studied collaboratively by Hildebrand (3) and by Redfern (4). Although Hildebrand (3) found the level of precision to be satisfactory when the original Ayre and Anderson techniques were employed, Redfern (4) suggested that work on the semi-autolytic method of Landis (2) be discontinued. Recently Miller (5) made a critical study of the modified Ayre and Anderson method and discussed the conditions which must be maintained to secure a high level of precision. Warshowsky and Geddes (6) performed a comparative study of the Ayre and Anderson method and the Modified Landis Procedure (2) and concluded that better differentiation between samples was afforded by the modified Ayre and Anderson procedure.

The present collaborative study was undertaken to test the applicability of the method as standardized by Miller (5) to several materials possessing a wide range of proteolytic activity and, further, to test the reliability of the method as used in several laboratories.

PROTEOLYTIC ACTIVITY OF FLOUR AND MALTED WHEAT FLOUR

(Applicable to slightly active materials such as patent flour or to diluted extracts of active proteolytic preparations.)

REAGENTS

(a) *Buffer stock soln.*—Make 120 ml of acetic acid and 164 g anhydrous sodium acetate up to 1000 ml with H₂O. Dilute 1:20 before using (pH 4.7).

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

† Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Milling Industry, Kansas State College. Published as Contribution No. 156, Department of Milling Industry, Kansas State College.

(b) *Bacto-hemoglobin substrate*.*

(c) *Trichloroacetic acid solns*:

Soln (1).—Dissolve 36 g trichloroacetic acid in 64 ml water. Use 5 ml aliquot.

Soln (2).—Dissolve 36 g trichloroacetic acid in 44 ml water. Use 4 ml aliquot.

(d) *Kjeldahl solns*.—Including 0.0714 *N* sodium hydroxide.

(e) *Pumice or fine sand*.

METHOD

Preparation of enzyme solutions.—For slightly active materials such as flour, weigh as much as 10 g directly into digestion flasks. For active enzyme preparations prepare a water extract or suspension immediately preceding digestion. (The amount of extract or dilutions thereof used in the digestion mixture may vary up to 1 ml. Appropriate activation technics may be applied to the enzyme extract.)

Digestion procedure.—Weigh 0.625 g (moisture-free basis) of Bacto-hemoglobin into each 125 ml Erlenmeyer flask and add ca 3 g of finely divided pumice. Add 5 g sample of flour to each of two flasks and agitate the mixture by rotation until flour and substrate are intimately mixed. Then add 25 ml of reagent (a) previously warmed to 40° (in thermostat-controlled bath within $\pm 0.1^\circ\text{C}$.) to each flask and agitate its contents to insure uniform suspension. Place the tightly stoppered flasks in a constant temp. (40°) bath and agitate either continuously or at hourly intervals.

Add a 5 ml portion of trichloroacetic acid soln c(1) to one flask of each pair at the end of 15 min. digestion and to the second flask of the pair after 5 hours of digestion. Mix contents thoroly and allow flasks to remain in the bath at 40°C. for exactly 30 min. Centrifuge the suspension for 5 min. at 1800 r.p.m. and filter. Pipet duplicate 5 ml aliquots directly into Kjeldahl flasks and determine soluble nitrogen.

Follow essentially the same procedure in determining the enzyme activity of an extract. In place of the solid material, use a total of 1 ml of extract or extract plus water. After zero time and 5 hour digestion periods, add to each flask a 4 ml aliquot of trichloroacetic acid soln c(2). Thoroly mix the contents, allow to remain in the water bath for exactly 30 min. and filter without centrifuging; analyze 5 ml aliquots for soluble nitrogen.

Soluble nitrogen.—Proceed as under 2.24, 2.25, or 2.26. Use a definite volume of water (350 ml) to dilute the cooled digest and add in such a way as to wash down all the trichloroacetic acid which has condensed in the neck of the flask during the digestion process. Also add the concentrated alkali (one and one-half times the usual quantity) in such a manner as to lave the neck of the flask. After distillation, back-titrate the unneutralized standard acid with 0.0714 *N* sodium hydroxide.

Expression of proteolytic activity.—Proteolytic activity is measured by the difference in back-titration volumes for the 15-min. or zero time digestions and the corresponding 5 hour digestion, expressed in ml of 0.0714 *N* sodium hydroxide. This difference may be translated into mg of soluble nitrogen released from a given weight of the enzyme source.

NOTES

(1) A duplicate Kjeldahl determination is made on each digestion filtrate and in most cases there should be enough sample left for a third analysis if one titration should be of doubtful accuracy. Duplicate titrations should vary by no more than 0.05 ml of 0.0714 *N* sodium hydroxide.

* A suitable quality is obtainable from the Difco Laboratories, Detroit, Mich.

(2) Each still should be previously checked for leaks and duplicability by distilling aliquots of ammonium oxalate solution to which 350 ml water and 10 ml concentrated sodium hydroxide have been added. A 5 ml aliquot containing 25 mg of ammonium oxalate is convenient.

(3) Higher concentrations of hemoglobin substrate should not increase the corrected titration values for the enzyme sources studied.

(4) Careful washing down of the trichloroacetic acid from the neck of the digestion flasks is mandatory.

(5) An enzyme such as papain can be activated by bubbling washed hydrogen sulfide gas through the enzyme suspension and through the digestion mixture itself.

(6) For some materials such as flour a turbid solution may remain after the final filtration. Such turbidity may be removed by boiling the centrifuged digestion mixture for a few seconds prior to final filtration. The liquid lost through evaporation should be replaced by the addition of water.

COLLABORATIVE STUDY

Preparation of Samples and Instructions to Collaborators.—Each of eight collaborators tested a total of four samples which included a patent flour, a malted wheat flour, a commercial fungal amylase concentrate, and a commercial papain preparation. All samples were thoroughly blended; sub samples were taken, sealed, and shipped. Included in the shipment were sufficient Bacto-hemoglobin, pumice, and concentrated buffer solution to complete the analyses.

The collaborators were requested to complete a set of blank and 5 hour digestions on three different days, which were not necessarily consecutive, and to make duplicate Kjeldahl nitrogen analyses on each filtered solution. The amount of sample for each digestion was fixed at 5 g for patent flour and malted wheat flour, 30 mg. for the fungal concentrate and 5 mg. for papain. Since most of the variability in the method appears to be associated with the Kjeldahl procedure it was suggested that someone thoroughly familiar with that technic run the nitrogen analyses and that the same individual be employed throughout the study.

Results and discussion.—Results obtained by two analysts were excluded at their own request. The individual results of each of the remaining six collaborators are summarized in Table 1. The means and standard deviations for each type of material are also included.

The low average values for papain, obtained by collaborators A and E are probably due to inactivation of the enzyme on standing. These data, therefore, were not analyzed statistically.

The analysis of variance shown in Table 2 for the remaining data indicates a significant difference in results obtained by different collaborators for malted wheat flour and the fungal concentrate. A non-significant difference, however, was obtained for patent flour. For digestions within the same laboratory significant differences are shown for all preparations.

Additional knowledge of the precision of the method can be gained by considering the work of one analyst. The results obtained over a period of

TABLE 1.—*Proteolytic activity of four different enzyme sources as determined by six collaborators*

Values expressed in ml of 0.0714 N sodium hydroxide

REPLICATE DIGESTIONS		PATENT FLOUR			MALTED WHEAT FLOUR			FUNGAL PREP.			PAPAIN		
COLLABORATOR	DAY												
		ml	ml	Av.	ml	ml	Av.	ml	ml	Av.	ml	ml	Av.
A	1	1.23	1.15		4.85	4.88		3.43	3.50		2.60	2.55	
	2	1.15	1.25		4.93	4.90		3.55	3.60		2.90	2.90	
	3	1.13	1.08		4.88	4.88		3.55	3.50		2.65	2.60	
				1.16			4.90			3.50			2.70
B	1	1.11	1.12		4.87	4.89		3.52	3.61		3.06	3.04	
	2	1.08	1.08		4.82	4.87		3.66	3.69		3.28	3.23	
	3	1.12	1.11		5.00	4.90		3.65	3.61		3.10	3.05	
				1.10			4.89			3.60			3.16
C	1	1.25	1.20		4.85	4.90		3.30	3.40		3.10	3.15	
	2	1.20	1.20		4.75	4.95		3.35	3.30		3.15	3.15	
	3	1.15	1.10		4.75	5.00		3.35	3.40		3.15	3.70	
				1.18			4.87			3.35			3.14
D	1	1.09	1.08		5.06	4.99		3.21	3.17		3.17	3.16	
	2	1.21	1.17		4.79	4.97		3.02	3.03		3.13	3.19	
	3	1.17	1.12		4.84	4.80		3.11	3.25		3.24	3.09	
				1.14			4.91			3.13			3.16
E	1	1.19	1.30		4.83	4.94		3.36	3.53		2.58	2.56	
	2	1.12	1.15		5.21	5.14		3.63	3.61		2.60	2.65	
	3	1.12	1.12		4.90	4.97		3.68	3.74		3.15	3.08	
				1.17			5.00			3.59			2.77
F	1	1.30	1.20		4.80	4.75		3.10	3.10		2.95	2.95	
	2	0.90	0.90		4.50	4.60		3.20	3.20		3.00	3.00	
	3	1.10	1.15		4.60	4.50		3.10	3.20		2.95	2.95	
				1.09			4.63			3.15			2.97
Mean, all samples		1.14			4.87			3.39			2.98		
Standard deviation within laboratory		0.08			0.10			0.08			—		

several months by one individual working with a fungal amylase preparation at various concentrations are reported in Table 3. Values for duplicate 5-hour digestions are recorded. The standard deviation of 0.04 ml calculated from these data is approximately one-half that calculated for the preparations analyzed collaboratively. This indicates that experience contributes substantially to a higher degree of precision of the method.

The standard error of a single determination as calculated by Hildebrand (3) from data obtained in a collaborative study of the Ayre and Anderson method was 0.99 mg of nitrogen per 5 grams of flour sample. The value obtained from the data in Table 3 is 0.24 mg for an equivalent

quantity of flour, or less than one-fourth the standard error reported previously. For the collaborative data in Table 1 the standard error for a single determination is approximately one-half as large as the value reported by Hildebrand (3).

TABLE 2.—*Analysis of variance of collaborative data*

(2) compared with (3) and (1) compared with (2).

VARIANCE DUE TO:	DEGREES OF FREEDOM	MEAN SQUARES		
		PATENT FLOUR	MALTED WHEAT FLOUR	FUNGAL CONCENTRATE
(1) Collaborators	5	.006	.0941**	.272***
(2) Between digestions in same laboratory	12	.015**	.0184**	.011**
(3) Between analyses within same digestion	18	.0021	.0058	.003

** Significance exceeds the 1% level.

*** Significance exceeds the 0.1% level.

TABLE 3.—*Replicability of proteolytic enzyme
assay within one laboratory*

Values expressed in ml of 0.0174 N sodium hydroxide

DIGESTION		ABSOLUTE DIFFERENCE	DIGESTION		ABSOLUTE DIFFERENCE
I	II		I	II	
<i>ml</i>			<i>ml</i>		
1.40	1.38	.02	2.80	2.80	.00
0.25	0.20	.05	0.35	0.40	.05
0.20	0.15	.05	0.25	0.40	.15
0.05	0.00	.05	1.75	1.73	.02
0.30	0.28	.02	1.48	1.50	.02
1.60	1.53	.07	1.00	1.10	.10
0.38	0.28	.10	0.30	0.30	.00
1.10	1.10	.00	1.65	1.65	.00
0.60	0.70	.10	1.05	1.05	.00
1.40	1.38	.02	0.90	0.85	.05
0.10	0.08	.02	0.05	0.05	.00
1.45	1.48	.03	1.18	1.20	.02
0.54	0.62	.08			
			Mean		.04
			Standard deviation		.04

Although significant tendencies were found for different laboratories to obtain different results and for one analyst to obtain significant differences between digestions, the modified Ayre and Anderson method (5) gives a lower level of error than the original Ayre and Anderson method (1). It is

believed that these data justify the recommendation that the method as written be officially adopted for the determination of the proteolytic activity of patent flour and malted wheat flour.

LIST OF COLLABORATORS

The letters assigned the collaborators in Table 1 bear no relation to the alphabetical arrangement in this list.

Allan D. Dickson, U. S. Department of Agriculture, Madison, Wis.
 John W. Giertz, Kansas Milling Company, Wichita, Kan.
 Rae H. Harris, North Dakota Agricultural Experiment Station, Fargo, N. D.
 B. D. Hites, Nebraska Agricultural Experiment Station, Lincoln, Neb.
 Eric Kneen, Kurth Malting Company, Milwaukee, Wis.
 Byron S. Miller, U. S. Department of Agriculture, Manhattan, Kan.
 Hugh K. Parker, Wallace and Tiernan Company, Newark, N. J.
 Roland W. Selman, C. J. Patterson Company, Kansas City, Mo.

COMMENTS OF COLLABORATORS

(1) The quantities of alkali and water suggested made the Kjeldahl distillations quite difficult to run. It was necessary to reduce the volume of alkali to 75 ml and the water to 250 ml. By carefully adding these solutions from large burettes no further difficulties were encountered.†

(2) The weighing, transferring and mixing of the 5-g samples of flour were found to be the most tedious requirements of this method.

(3) From our work we would judge the method to be satisfactory for the determination of proteolytic activity and feel no need for major modifications.

(4) Some difficulty was encountered with the 5-hour digestion of patent flour. The filtrate was cloudy after centrifuging and filtering. Replication was poorer on this sample than for the others.

RECOMMENDATIONS*

It is recommended—

(1) That the method as proposed be officially adopted for the determination of proteolytic enzymes in patent flour and malted wheat flour.

(2) That further work be done on the application of the method to the analysis of proteolytic enzymes in materials other than patent flour and malted wheat flour.

(3) That other methods for the determination of proteolytic activity be investigated.

ACKNOWLEDGMENTS

The Associate Referee gratefully acknowledges and appreciates the co-operation manifested by all collaborators and their respective organizations. His thanks are also extended to Mr. B. Marlo Dirks for the use of the data included in Table 3 and to Prof. H. C. Fryer, Head of the Statistical Laboratory, Kansas State College, for consultation on the statistical aspects of the problem.

† The volumes of solutions suggested in the procedure are for 800 ml Kjeldahl flasks.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

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REPORT ON ANALYSIS OF SOYBEAN FLOUR

By W. L. TAYLOR (General Mills, Inc., Minneapolis, Minnesota),
Associate Referee

The changes made in the tentative method for soybean flour* at the 1947 meeting of the Association indicated the need of further collaborative

TABLE 1.—*Collaborative results*

LABORATORY	MOISTURE	PROTEIN (N × 6.25)	OIL	ASH
	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
		<i>Defatted Soy Flour</i>		
A	(7.94) (7.82) 7.88	(55.25) (55.15) 55.20	(.69) (.72) .705	(6.02) (6.09) 6.05
B	(8.02) (7.94) (7.90) 7.95	(55.38) (55.62) — 55.50	(.674) (.658) — .666	(6.02) (6.09) (6.13) 6.08
C	(7.76) (7.73) 7.74	(54.2) (54.6) 54.40	(.66) (.67) .665	(6.16) (6.11) 6.13
		<i>Low Fat Soy Flour</i>		
A	(6.23) (6.33) 6.28	(51.45) (51.45) 51.45	(5.21) (5.21) 5.21	(5.77) (5.90) 5.83
B	(6.48) (6.61) (6.52) 6.54	(51.125) (51.187) — 51.16	(5.35) (5.34) — 5.34	(5.82) (5.80) (5.83) 5.82
C	(6.43) (6.41) 6.42	(50.6) (50.4) 50.50	(5.41) (5.41) 5.41	(5.63) (5.82) 5.82
		<i>Full Fat Soy Flour</i>		
A	(6.39) (6.34) 6.37	(44.40) (44.50) 44.45	(20.20) (20.07) 20.14	(4.54) (4.64) 4.59
B	(6.61) (6.62) (6.42) 6.48	(44.50) (44.87) — 44.69	(19.63) (19.67) — 19.65	(4.68) (4.73) (4.83) 4.75
C	(6.16) (6.09) 6.12	(43.8) (43.8) 43.80	(20.3) (20.1) 20.20	(4.68) (4.70) 4.69

work. Supplies of the three leading types of soy flour, namely, defatted soy flour, low fat soy flour, and full fat soy flour were, therefore, obtained from manufacturers. From these supplies one pound samples were prepared and distributed to four collaborators (three of whom completed the work, with one incomplete and too late to tabulate). The collaborators were directed to follow the tentative method for moisture, 20.4, nitrogen 2.26, oil, 31.67, and ash 27.9, as revised at the 1947 meeting.*

COLLABORATORS

Randall, Fred, Cooperative G. L. F. Mills, Inc., Buffalo, N. Y.

Blanchard, J. F., Department of Health and Welfare, Food and Drugs Office, Winnipeg, Manitoba, Canada.

Kyser, George H., General Mills, Inc., Chemical Division, Belmond, Iowa.

DISCUSSION

One of the reasons for doing the collaborative work reported above is that few laboratories have been called upon to make routine analyses of soy flours. It is, therefore, more necessary than usual to use methods which can be followed exactly by any competent chemist and to prescribe the reagents and equipment needed for making the determinations.

RECOMMENDATION†

In order that the tentative method for the analysis of soy flour may be thoroughly tested, it is recommended that the collaborative work on soy flour be continued and that the assistance of additional laboratories be enlisted.

No reports were given on starch in raw and cooked cereals; fat acidity in grain; sugar in bread, flour, etc.; soybean flour in foods (immunological test); baked products (moisture, ash, protein, fat, and crude fiber); moisture in self-rising flour, etc.; bromates in flour; phosphorus; unsaponifiable matter and sterols in noodles, etc.; and carbon dioxide in self-rising flours.

REPORT ON BAKING POWDER

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

The Associate Referee has submitted a report of his collaborative study on the application of the A.O.A.C. tentative method and 3 modifications of drying oven methods to three samples of baking powder for determination of residual carbonic acid. The results obtained warrant the recom-

* See *This Journal*, 31, 81 (1948).

† For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

mentation of the Associate Referee that the A.O.A.C. tentative method be adopted as official, first action, in which the Referee concurs.

The results obtained by the oven modification No. 1 method (heated 5 hrs. at 100°C. with 20 ml added water) were as good as those obtained by the A.O.A.C. method. In view of the need for a special steam bath for the A.O.A.C. tentative method and the common existence of an accurate electric oven in all laboratories, it is recommended that the oven modification No. 1 method be adopted as an alternate method, official first action, in which the Associate Referee concurs.

In the determination of available carbon dioxide in baking powder, the present official method for total carbon dioxide specifies sulfuric acid (1+5), and the official (first action) residual carbon dioxide methods (recommended above) specify hydrochloric acid (1+2). There are certain definite advantages to the use of hydrochloric acid (1+2), especially for baking powder containing added calcium carbonate. In a collaborative report by J. R. Chittick, *This Journal*, 29, 259, it is concluded that hydrochloric acid (1+2) be an alternate for sulfuric acid (1+5) in the official method of total carbon dioxide.

There are official methods for starch in baking powder, and there is no general need for the tentative, modified McGill method, 17.21.

RECOMMENDATIONS*

It is recommended—

- (1) That the tentative, modified McGill method, 17.21 be dropped.
- (2) That the tentative qualitative test for phosphoric acid, 17.31, be adopted as official, first action.
- (3) That the tentative methods, *This Journal*, 31, 78, and the oven modification No. 1, as set forth in the Associate Referee report for this year on residual CO₂ in baking powder, be adopted as official, first action.
- (4) That HCl (1+2), be adopted as alternate for H₂SO₄ (1+5) in the official method for total CO₂, 17.4 and 17.6.
- (5) That the available CO₂, 17.9, be determined by subtraction of residual CO₂ (see recommendation 3) from the total CO₂, 17.6.
- (6) That the official gasometric method 17.8 be dropped, final action.

REPORT ON RESIDUAL CARBON DIOXIDE IN BAKING POWDERS

By J. E. TATAR (Standard Brands Inc., Chicago, Ill.),
Associate Referee

This report covers a continuation of the collaborative study of the Quartermaster Corps method (Rewritten Version) which was adopted as

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 62 (1949).

a tentative method for the determination of residual carbon dioxide in baking powders,¹ and modifications of this method as outlined in the recommendations of the Associate Referee in his last report.²

One of the recommendations calls for a study of the tentative method in which the evaporation to dryness is carried out in a drying oven instead of a water bath and at temperatures ranging from 70 to 100°C. The lower temperatures were suggested to effect the evaporation to dryness without gelatinization of the starch which may interfere both in the driving out of the available CO₂ during the treatment of the sample, and the shaking out of the residual CO₂ in the final determination. Preliminary work done in the Associate Referee's laboratory indicated that although gelatinization of the starch takes place on the water bath, it does not proceed in the oven to an extent great enough to interfere until temperatures of over 100°C. are reached. Therefore, the work with the drying oven methods was limited to temperatures of 90 and 100°C.

Another recommendation which was made in the Associate Referee's report and which was tried in his laboratory before submitting it for collaborative study, was the use of a saturated sodium chloride solution as a reaction medium instead of distilled water. It was found that the evaporation to dryness took much too long whether it was done on the water bath or in the oven, and any other treatment short of evaporation to dryness did not drive out all of the available CO₂. It could be accomplished by boiling, but it was difficult to standardize and control the technique. Therefore, no collaborative work was done on this recommendation.

Four methods were submitted for collaborative study:

The A.O.A.C. Tentative method, *This Journal*, 31, 78 (1948).

Drying Oven Modification No. 1—100°C., with 20 ml. H₂O.*

Drying Oven Modification No. 2—100°C., with 10 ml H₂O.

Drying Oven Modification No. 3—90°C., with 10 ml H₂O.

The four methods were applied to three baking powders, the formulas of which were exactly the same as in last year's work.³ They were marked D, E, and F, corresponding to A, B, and C, respectively of last year's samples. However, the ingredients used in making up the baking powder samples were not all from the same lots as last year's samples, and for that reason powders D and E had slightly lower residuals.

Collaborators were requested to report on all four methods, running each sample once a day on three different days, instead of in triplicate on the same day, so that the results would reflect a more accurate measure

¹ See report of Referee, *This Journal*, 31, 273 (1948).

² See report of Associate Referee, *This Journal*, 31, 274 (1948).

* Adopted as an alternate method, *This Journal*, 32, 83 (1949).

³ See report of Associate Referee, *This Journal*, 31, 275 (1948).

TABLE 1.—Results of collaborators on sample "D" in percent carbon dioxide

ANALYST	TENTATIVE A.O.A.C. METHOD	DEVIATION FROM MEAN	OVEN MODIFICA- TION #1	DEVIATION FROM MEAN	OVEN MODIFICA- TION #2	DEVIATION FROM MEAN	OVEN MODIFICA- TION #3	DEVIATION FROM MEAN
1	0.80	0.24	1.00	0.23	0.70	0.05	0.90	0.19
	0.71	0.15	.91	0.14	0.81	0.16	0.91	0.20
	0.70	0.14	.80	0.03	0.80	0.15	1.10	0.39
2	0.58	0.02	0.99	0.22	0.71	0.06	0.76	0.05
	0.68	0.12	0.97	0.20	0.68	0.03	0.95	0.24
	0.60	0.04	0.80	0.03	0.70	0.05	0.82	0.11
3	0.52	0.04	0.70	0.07	0.51	0.14	0.56	0.15
	0.52	0.04	0.57	0.20	0.46	0.19	0.51	0.20
	0.40	0.16	0.61	0.16	0.61	0.04	0.61	0.10
4	0.55	0.01	0.83	0.06	0.66	0.01	0.70	0.01
	0.50	0.06	0.85	0.08	0.70	0.05	0.60	0.11
	0.51	0.05	0.78	0.01	0.71	0.06	0.62	0.09
5	0.30	0.26	0.60	0.17	0.50	0.15	0.50	0.21
	0.30	0.26	0.65	0.12	0.49	0.16	0.64	0.07
	0.39	0.17	0.68	0.09	0.44	0.21	0.60	0.11
6	0.74	0.18	0.82	0.05	0.99	0.34	0.97	0.26
	0.78	0.22	0.84	0.07	1.06	0.41	0.96	0.25
	0.77	0.21	0.86	0.09	0.97	0.32	0.98	0.27
7	0.77	0.21	0.73	0.04	0.76	0.11	0.82	0.11
	0.71	0.15	0.73	0.04	0.81	0.16	0.73	0.02
	0.72	0.16	0.77	0.00	0.72	0.07	0.77	0.06
8	0.64	0.08	0.67	0.10	0.43	0.22	0.73	0.02
	0.61	0.05	0.87	0.10	0.31	0.34	0.61	0.10
	0.61	0.05	0.93	0.16	0.77	0.12	0.62	0.09
9	0.40	0.16	0.62	0.15	0.61	0.04	0.51	0.20
	0.62	0.06	0.64	0.13	0.61	0.04	0.62	0.03
	0.49	0.07	0.90	0.13	0.61	0.04	0.69	0.04
10	0.30	0.26	0.59	0.18	0.52	0.13	0.62	0.03
	0.29	0.27	0.65	0.12	0.52	0.13	0.47	0.18
	0.37	0.19	0.65	0.12	0.44	0.21	0.47	0.18
Mean	0.56		0.77		0.65		0.71	
Ave. Dev.		0.14		0.11		0.14		0.14
Max. Var.	0.51		0.43		0.75		0.63	

TABLE 2.—*Results of collaborators on sample "E" in percent carbon dioxide*

ANALYST	TENTATIVE A.O.A.C. METHOD	DEVIATION FROM MEAN	OVEN MODIFICA- TION #1	DEVIATION FROM MEAN	OVEN MODIFICA- TION #2	DEVIATION FROM MEAN	OVEN MODIFICA- TION #3	DEVIATION FROM MEAN
1	6.91	0.34	7.01	0.41	6.91	0.16	6.91	0.13
	6.97	0.40	6.97	0.37	7.07	0.32	7.07	0.29
	6.90	0.33	6.90	0.30	6.90	0.15	6.90	0.12
2	6.41	0.16	6.59	0.01	6.50	0.25	6.74	0.04
	6.59	0.02	6.78	0.18	6.65	0.10	6.90	0.12
	6.60	0.03	6.59	0.01	6.80	0.05	6.83	0.05
3	6.45	0.12	6.62	0.02	6.72	0.03	6.75	0.03
	6.45	0.12	6.59	0.01	6.74	0.01	6.89	0.11
	6.40	0.17	6.51	0.09	6.83	0.08	6.81	0.03
4	6.64	0.07	6.53	0.07	6.67	0.08	6.77	0.01
	6.63	0.06	6.55	0.05	6.65	0.10	6.60	0.18
	6.50	0.07	6.44	0.16	6.75	0.00	6.73	0.05
5	6.22	0.35	6.59	0.01	6.58	0.17	6.61	0.17
	6.29	0.28	6.46	0.14	6.56	0.19	6.80	0.02
	6.42	0.15	6.44	0.16	6.56	0.19	6.56	0.22
6	6.61	0.04	6.52	0.08	7.21	0.46	7.09	0.31
	6.63	0.06	6.55	0.05	7.26	0.51	7.14	0.36
	6.60	0.03	6.54	0.06	7.19	0.44	7.24	0.46
7	6.74	0.17	6.64	0.04	6.70	0.05	6.87	0.09
	6.61	0.04	6.59	0.01	6.70	0.05	6.79	0.01
	6.77	0.20	6.57	0.03	6.72	0.03	6.87	0.09
8	6.63	0.06	6.46	0.14	6.55	0.20	6.49	0.29
	6.49	0.18	6.86	0.26	6.86	0.11	6.72	0.06
	6.60	0.03	6.67	0.07	6.91	0.16	6.87	0.09
9	6.64	0.07	6.49	0.11	6.68	0.07	6.73	0.05
	6.61	0.04	6.52	0.08	6.69	0.06	6.69	0.09
	6.65	0.08	6.60	0.00	6.85	0.10	6.76	0.02
10	6.42	0.15	6.49	0.11	6.50	0.25	6.42	0.36
	6.42	0.15	6.41	0.19	6.38	0.37	6.53	0.25
	6.34	0.23	6.40	0.20	6.55	0.20	6.45	0.33
Mean	6.57		6.60		6.75		6.78	
Ave. Dev.		0.14		0.11		0.16		0.15
Max. Var.	0.75		0.57		0.88		0.82	

TABLE 3.—Results of collaborators on sample "F" in percent CO_2

ANALYST	TENTATIVE A.O.A.C. METHOD	DEVIATION FROM MEAN	OVEN MODIFICA- TION #1	DEVIATION FROM MEAN	OVEN MODIFICA- TION #2	DEVIATION FROM MEAN	OVEN MODIFICA- TION #3	DEVIATION FROM MEAN
1	0.50	0.16	0.50	0.15	0.50	0.17	0.50	0.19
	0.51	0.17	0.51	0.16	0.51	0.18	0.51	0.20
	0.50	0.16	0.50	0.15	0.50	0.17	0.50	0.19
2	0.39	0.05	0.52	0.17	0.38	0.05	0.38	0.07
	0.34	0.00	0.39	0.04	0.39	0.06	0.34	0.03
	0.38	0.04	0.40	0.05	0.36	0.03	0.40	0.09
3	0.42	0.08	0.30	0.05	0.10	0.23	0.25	0.06
	0.42	0.08	0.21	0.14	0.31	0.02	0.31	0.00
	0.35	0.01	0.25	0.10	0.31	0.02	0.31	0.00
4	0.39	0.05	0.41	0.06	0.30	0.03	0.23	0.08
	0.38	0.04	0.28	0.07	0.30	0.03	0.20	0.11
	0.40	0.06	0.32	0.03	0.34	0.01	0.33	0.02
5	0.10	0.24	0.30	0.05	0.20	0.13	0.10	0.21
	0.20	0.14	0.30	0.05	0.20	0.13	0.20	0.11
	0.30	0.04	0.39	0.04	0.20	0.13	0.20	0.11
6	0.15	0.19	0.18	0.17	0.34	0.01	0.29	0.02
	0.18	0.16	0.20	0.15	0.32	0.01	0.30	0.01
	0.22	0.12	0.21	0.14	0.38	0.05	0.32	0.01
7	0.31	0.03	0.41	0.06	0.46	0.13	0.41	0.10
	0.31	0.03	0.36	0.01	0.41	0.08	0.36	0.05
	0.36	0.02	0.46	0.11	0.36	0.03	0.36	0.05
8	0.41	0.07	0.33	0.02	0.30	0.03	0.31	0.00
	0.31	0.03	0.46	0.11	0.43	0.10	0.30	0.01
	0.51	0.17	0.49	0.14	0.51	0.18	0.33	0.02
9	0.33	0.01	0.34	0.01	0.40	0.07	0.31	0.00
	0.42	0.08	0.36	0.01	0.33	0.00	0.26	0.05
	0.41	0.07	0.41	0.06	0.36	0.03	0.45	0.14
10	0.16	0.18	0.29	0.06	0.10	0.23	0.15	0.16
	0.19	0.15	0.31	0.04	0.23	0.10	0.18	0.13
	0.32	0.02	0.18	0.17	0.13	0.20	0.19	0.12
Mean	0.34		0.35		0.33		0.31	
Ave. Dev.		0.09		0.09		0.09		0.08
Max. Var.	0.41		0.34		0.41		0.41	

of the precision. The assistance of the following collaborators is gratefully acknowledged:

Barackman, R. A., and Gilkey, D. L., Victor Chemical Works, Chicago Heights, Ill.

Bryan, C. S., and Randall, H. C., Rumford Chemical Works, Rumford, R. I.
Dick, Ludwig, Standard Brands, Inc., Chicago, Ill.

Holch, R. D., and Martin, W. T., Jaques Mfg. Co., Chicago, Ill.

Morek, R. A., R. B. Davis Co., Hoboken, N. J.

Munsey, V. E., and Steagall, Edward, Food and Drug Administration, Washington, D. C.

Novitsky, Peter, and Matson, Gloria, General Foods Corporation, Chicago, Ill.

Pugsley, L. I., and Kelly, J. T., Food and Drug Division, Ottawa, Canada.

Schilb, T. W., and McKim, Elizabeth, Monsanto Chemical Co., St. Louis, Mo.

Steele, Harold K., Fleischmann Laboratories, New York, N. Y.

(The order in which these collaborators are listed does not conform to the numbers used in the tables of results.)

DISCUSSION

The results shown in Tables 1, 2, and 3 reflect a definite improvement in the agreement among the collaborators when compared to last year's collaborative study. This is particularly true of the results obtained with the tentative method on baking powder E which contained calcium carbonate and which corresponded to baking powder B in last year's work. The maximum variance for this powder with the tentative method last year was 1.95 compared to 0.75 in this year's results and an average deviation of 0.37 last year compared to 0.14.

All four methods were equally good on powder F, the average with the tentative method (0.34%) being a perfect check of last year's average for the same powder, while the averages for this powder with the other three methods were 0.35, 0.33, and 0.31, also excellent checks.

With powders D and E the tentative method and the No. 1 oven modification were superior to the other two in having lower maximum variances and the average deviations and maximum variances obtained with the No. 1 oven modification on those powders gave this method a slight edge in precision over the tentative method. However, this method requires 5 hours of oven drying time compared to 2 hours on the water bath for the tentative method, and the differences in precision are not large enough to compensate for the extra time. Neither of the two methods require much of the operator's time, so that they are equal from that standpoint. The oven modification requires a thermostatically controlled drying oven, whereas the tentative method can be carried out on a simple water bath heated by gas or electricity.

RECOMMENDATIONS*

It is recommended—

That the tentative method and the Drying Oven Modification No. 1, *This Journal*, 32, 83, (1949), be adopted as official, first action, for the determination of residual CO₂ in baking powders, and that the study be discontinued.

REPORT ON PLANTS

By ELROY J. MILLER (Michigan Agricultural Experiment Station,
East Lansing, Michigan) *Referee*

Substantial progress has been made by the Associate Referees on Plants during the past year. A number of new Associate Referees were appointed and have begun work on the methods assigned them for study. Several have reports to present.

The following have submitted reports:

E. J. Benne, Associate Referee on Carotene and Zinc in Plants, has a report on Zinc.

Gordon H. Ellis, Associate Referee, has a report on Cellulose and Lignin in Plants.

Ray L. Shirley, Associate Referee on Sodium, has a report on that subject.

Carroll L. Hoffpauir, Associate Referee on Starch, has a report on Starch in Plants.

L. K. Wood, Associate Referee on Boron, has a report in collaboration with C. M. Austin.

All the Associate Referees expect to continue work on their respective methods for constituents in plants.

RECOMMENDATIONS†

It is recommended—

(1) That the resignation of J. S. McHargue, Associate Referee on Boron and Iodine, be accepted with appreciation for his long and valuable service.

(2) That the resignation of Mrs. Dorothy Waldron be accepted with thanks for past contributions.

(3) That the list of Associate Referees and their assignments as given in *This Journal*, 31, 284 (1948), be continued.

(4) That the recommendations made by the Associate Referees in their Reports on Zinc, Boron, Cellulose and Lignin, Sodium and Starch, respectively be accepted.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 62 (1949).

† For report of Subcommittee A and action of the Association, see *This Journal*, 32, 45 (1949).

REPORT ON ZINC IN PLANTS

By RAY L. SHIRLEY, ERWIN J. BENNE (*Associate Referee*), and E. J. MILLER (Michigan Agricultural Experiment Station, East Lansing, Michigan)*

The tentative A.O.A.C. method for determining zinc in plant materials (12.24) (1) depends upon the combination of zinc with dithizone (diphenylthiocarbazone) under controlled conditions. The zinc dithizonate and some excess dithizone are removed from an ammoniacal aqueous solution with carbon tetrachloride, and the zinc is evaluated photometrically by a mixed-color procedure. This method was published by Cowling and Miller (2) and was accepted as a tentative method by the Association in 1941 upon the recommendation of Cowling (3), who was then Associate Referee on zinc in plants.

Shirley *et al.*, investigated this method and a report of the findings was presented at the annual meeting of the Association in 1947 and later published (4). The present authors have continued this investigation, and this report presents a modification of the tentative procedure which possesses the following advantages: (a) one extraction step preliminary to the extraction of the zinc dithizonate is eliminated, (b) two less reagents are necessary, (c) only one separatory funnel is required per determination, and (d) about one-third less time is required for making an analysis.

MODIFIED METHOD

APPARATUS

The same apparatus as is used in the tentative method except that amber or low actinic glassware is preferable for certain operations (4).

REAGENTS

The same as required in the tentative method except that reagent B and 0.02 *N* HCl are no longer used.

PROCEDURE

Ashing and Extraction of Ash

Weigh 5 gms. of finely-ground, air-dried plant material into a platinum dish of suitable size. Mix 25 ml of ca *N* H₂SO₄ soln with the sample in the dish and dry on steam bath until most of the water is removed. Place the dish in hot air oven at 105°C. and complete removal of water. Ash in an electric muffle furnace at 500-550°C. and proceed as directed in the tentative procedure as far as "First Extraction."

Removal of Interferences, Formation of Zinc Dithizonate and Separation of Excess Dithizone

Add first 15 ml of distilled H₂O, then an aliquot of the ash extract containing not more than 30 mmg. of zinc, to an amber glass separatory funnel of 125 ml capacity.

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A 10 ml aliquot is satisfactory for most plant tissues, but if it is necessary to use a different volume, add 1 ml of 0.2 *N* HCl for each 5 ml of ash extract less than, or 1 ml of 0.2 *N* NH₄OH for each 5 ml more than, 10 ml taken, since it is essential that the soln have a *pH* of 2.0-2.3.

Add 10 ml of dithizone reagent to the separatory funnel, stopper funnel, and shake contents vigorously for 1 min. Allow the layers to separate and draw off and discard the CCl₄ layer. (Allow at least 3 min. for phases to separate after each shaking period thruout this procedure.) Add 6-8 ml of CCl₄ to the separatory funnel, stopper, and shake contents vigorously for 15 sec. Allow the layers to separate, shake down the CCl₄ from the surface, and draw off and discard the non-aqueous layer. Repeat this rinsing process twice more. After the last rinsing let the funnel stand long enough for all remaining CCl₄ to evaporate from the surface of the aqueous layer.

To the aqueous soln in the funnel add with a graduated cylinder 40 ml of ammonium citrate buffer soln and follow this with additions of exactly 5 ml of carbamate reagent and 10 ml of dithizone reagent from accurate pipets or burets. Stopper funnel and shake contents vigorously for 1 min. Allow the layers to separate and shake down CCl₄ from the surface. Draw off the aqueous layer thru a glass tube with a fine tip attached with rubber tubing to a water aspirator. Add 50 ml of 0.01 *N* NH₄OH, stopper funnel, and shake contents vigorously for 30 sec. to separate excess dithizone from the CCl₄ soln of zinc dithizonate.

Evaluation of Zinc Present

Draw off 2-3 ml of the CCl₄ soln of zinc dithizonate to flush out the bore of the stopcock and stem of the funnel and discard this portion. Collect the remainder in an amber glass bottle and stopper immediately. Pipet a 5-ml aliquot of this soln into a 25 ml, glass-stoppered volumetric flask of amber glass, make to volume with CCl₄, and mix.

Measure the per cent of light transmitted by the soln of zinc dithizonate with a suitable photoelectric colorimeter (or spectrophotometer) as directed in the A.O.A.C. procedure, and evaluate the quantity of zinc present from a light transmission-concentration curve prepared with known amounts of zinc carried through the modified procedure.

ACCURACY OF THE MODIFIED METHOD

The accuracy of the modified method was tested by: (a) analyzing synthetic solutions containing known amounts of zinc and various other cations, (b) analyzing several plant tissues with it and comparing values for zinc with those obtained by use of the A.O.A.C. method, and (c) analyzing plant tissues with and without added zinc. The results obtained are given in Tables 1, 2, and 3, respectively.

DISCUSSION

The addition of sulphuric acid to the sample prior to ashing was found to be advantageous in three ways: (a) reproducibility of results with some materials was improved, (b) it should help to prevent volatilization of zinc and many of its salts, and (c) removal of the ash from the platinum dishes during the extraction process was greatly facilitated.

Extraction of acid-stable dithizonates from an acidified, aqueous solution with a CCl₄ solution of dithizone has been practiced by numerous

investigators, including Fischer and Leopoldi (5), Hibbard (6), and Bendix and Grabenstetter (7). The results of this investigation show that interferences by other ions present in extracts of plant ash are as effectively eliminated by extracting directly from the acidified solution as by using the extra step specified in the A.O.A.C. method.

The data in Tables 1 and 2 indicate good reproducibility of results by

TABLE 1.—*Application of the modified procedure to the determination of zinc in solutions containing known amounts of zinc and various other cations*

Zn	MICROGRAMS							VARIATION FROM THE THEORETICAL
	Pb	Cu	Hg	Co	Cd	Ni	ZINC DETERMINED	
—	25	—	—	—	—	—	0	0
14.9	25	—	—	—	—	—	15.0	0.1
14.9	100	—	—	—	—	—	15.0	0.1
—	—	25	—	—	—	—	0	0
14.9	—	25	—	—	—	—	15.1	0.2
14.9	—	100	—	—	—	—	16.2	1.3
9.0	—	—	5	—	—	—	8.8	-0.2
14.4	—	—	100	—	—	—	14.5	0.1
9.6	—	—	—	—	—	5	10.4	0.8
9.6	—	—	—	—	—	10	10.3	0.7
20.2	—	—	—	—	—	25	21.1	0.9
9.6	—	—	—	5	—	—	9.2	-0.4
9.6	—	—	—	10	—	—	9.4	-0.2
9.6	—	—	—	20	—	—	9.7	0.1
9.0	—	—	—	—	2.5	—	9.5	0.5
20.2	—	—	—	—	100.0	—	24.0	3.8

TABLE 2.—*P.p.m. of zinc determined by the A.O.A.C. and modified procedures, respectively*

PLANT TISSUE ANALYZED	PROCEDURE	
	A.O.A.C. ¹	MODIFIED ²
Alfalfa leaf meal	26.9	28.0
Parsnip roots	22.2	24.6
Spinach leaves	45.1	42.4

¹ Averages of 8 results.

² Averages of 2 results.

the modified method. Consideration of the data in Table 3 is of interest. One hundred micrograms of lead in the aliquot analyzed did not interfere with the determination of zinc. Likewise 25 micrograms of copper did not interfere, but 100 micrograms gave a high value for zinc. In the authors' opinion, however, it seems that the latter quantity of copper would seldom

occur in the aliquot of an extract of plant ash suitable for the determination of zinc by this procedure. Out of 39 different kinds of plant materials that have been analyzed for copper in the authors' laboratory, the highest values obtained were 52 and 70 p.p.m. The remaining 37 values ranged from 1 to 30 p.p.m. Even with the tissue that contained 70 p.p.m. of copper, the aliquot normally used for determining zinc would contain only 35 micrograms, an amount that would probably be effectively removed by the modified procedure. In analyzing a sample for zinc that was known to

TABLE 3.—Zinc determined by the modified procedure in plant tissues with and without added zinc

PLANT TISSUE ANALYZED	MICROGRAMS OF ZINC IN ALIQUOTS ANALYZED				
	IN TISSUE ¹	ADDED	TOTAL PRESENT	DETERMINED ²	PER CENT OF TOTAL
Lettuce leaves	14.3	1.2	15.5	15.2	98.1
Lettuce leaves	14.3	2.4	16.7	16.3	97.3
Lettuce leaves	14.3	2.9 ³	17.2	16.6	96.5
Lettuce leaves	14.3	4.8 ³	19.1	19.6	102.4
Lettuce leaves	14.3	6.7 ³	21.0	21.9	104.3
Lettuce leaves	14.3	9.6 ³	23.9	24.5	102.5
Alfalfa leaf meal	11.6	4.8	16.4	15.9	96.7
Alfalfa leaf meal	11.6	9.6	21.2	21.3	100.5
Spinach leaves	11.8	4.8	16.6	17.0	102.4
Spinach leaves	11.8	9.6	21.4	21.2	99.1

¹ Determined by the modified procedure.

² Averaged values from replicate determinations

³ In these cases zinc was added after ashing, in all others before.

contain an above-average quantity of copper, a prolonged period of shaking during extraction of the acidified solution, as recommended by Bendix and Grabenstetter (7), would probably be effective in removing the copper. In the authors' experience an extra extraction with dithizone solution will achieve the same result.

The difference between the theoretical and determined values for zinc in the presence of 100 micrograms of mercury was within the limits of experimental error. Added nickel increased the determined value for zinc perceptibly; however, Bertrand and Makragnatz (8) determined nickel in a considerable variety of plant materials, and the highest concentration of nickel encountered was 3.5 p.p.m. Hence, it appears that nickel would seldom interfere seriously with the determination of zinc in plant material by the modified procedure. Cobalt up to 20 micrograms did not interfere with the determination. Out of 39 different kinds of plant materials that have been analyzed for cobalt in the authors' laboratory, the highest con-

centration encountered was .55 p.p.m.; therefore, it seems unlikely that cobalt would ever interfere to an appreciable extent in determining zinc.

Even small amounts of cadmium interfered perceptibly. Cholak *et al.* (9) attributed such interference to the instability of cadmium carbamate. Decomposition of this compound permits some of the cadmium to form a dithizonate which is evaluated as zinc. It should be pointed out, however, that this is true of the A.O.A.C. method also. Fortunately, high concentrations of cadmium are not known to occur in plants. Klein and Wichmann (10) analyzed several kinds of plant products and found less than 1 p.p.m. of cadmium. This is in keeping with the authors' experience in this regard.

It is believed that the modification of the tentative A.O.A.C. method presented in this report is definitely more convenient than the original and that it can be used without sacrifice of accuracy.

It is recommended* that the study be continued.

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REPORT ON SODIUM IN PLANTS

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In 1935 the Association accepted the magnesium uranyl acetate method as a tentative method for the determination of sodium in plant materials, and included it in the Plant Chapter of the Fourth Edition of *Methods of Analysis*, A.O.A.C. This method was proposed by Caley and Foulk (1) and is based on the precipitation of a salt formed between sodium and magnesium uranyl acetate.

* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 45 (1949).

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In 1934, Butler, then Associate Referee on sodium in plants, compared the method with the direct weight method and the sodium perchlorate method, in the analysis of a standard solution of sodium chloride; and with the latter when applied to the analysis of grasses and soil. At the time recommendation for adoption was made (3) as well as the following year (4) further study of the tentative method was recommended; however, no report of the proposed study has been published. The present Associate Referee was appointed in 1948 to carry out such a study, and the findings to date are described in this report.

EXPERIMENTAL AND RESULTS

To date the investigation has included the following: (A) A comparison of the tentative A.O.A.C. method (5) with the zinc uranyl acetate method of Barber and Kolthoff (6) in regard to (a) the solubility characteristics of the respective precipitates concerned and (b) the results obtained in the analysis of synthetic solutions and various crop plants; (B) The precision of the tentative method when applied to different plant materials and when varying sample weights were used; and (C) Application of the tentative method to the analysis of different plant materials with and without added sodium. Each of the above will be described in turn.

Effects of temperature on the solubility of sodium magnesium uranyl acetate, and the corresponding zinc salt, in the respective precipitating and wash reagents.—In this study approximately 0.1 g samples of sodium magnesium uranyl acetate and the corresponding zinc salt were weighed into 100 ml beakers, and 50 ml of the corresponding uranyl acetate reagent added and stirred at intervals to effect solution. In the case of the magnesium uranyl acetate reagent, studies were made on (a) reagent which was saturated previously at 23°C. with sodium magnesium uranyl acetate, and (b) reagent which was not saturated with the sodium salt. In the case of the zinc uranyl acetate it was saturated with the respective sodium salt but was used (a) with 15 ml of concentrated nitric acid per 100 ml of reagent added according to the recommendations of Broadfoot and Browning (7), and (b) without addition of nitric acid to it. Solubility determinations were made at 4°, 23°, and 35°C. The determinations at 4°C. were allowed to stand 44 hours in an ice box; whereas those made at 23° and 35° were allowed to be in contact with the salts for 30 minutes, after which the solutions were filtered through Sela crucibles. Fifteen ml of 95 per cent alcohol at the respective temperatures were used for transferring and washing the precipitates. The crucibles and contents were dried at 100°C. for 16 hours. The data obtained are shown in Table 1. These data indicate (a) that saturation of the magnesium uranyl acetate reagent with sodium magnesium uranyl acetate has little influence on the results obtained, (b) temperatures appreciably different from 23°C. which was the temperature at which the reagents were prepared should be avoided during the precipi-

tation of the sodium, and (c) the zinc uranyl acetate reagent with nitric acid is more sensitive to temperature changes in effecting solubility than is the magnesium uranyl acetate reagent.

TABLE 1.—*Effect of temperature on solubility of magnesium and zinc sodium uranyl acetate salts in their respective reagent solutions*

TEMP. C°	Mg ¹ PER 100 ml			
	MAGNESIUM SALT		ZINC SALT	
	SATURATED ²	UNSATURATED	WITH HNO ₃	WITHOUT HNO ₃
4 ³	-8.5	-10.8	-151.6	—
23	0.4	0.6	3.8	2.4
35	12.7	13.6	23.3	—

¹ Values given are averages of duplicate determinations.

² Saturated with sodium magnesium uranyl acetate at 23°C.

³ Negative signs indicate that salts came out of solution.

Solubility of magnesium and zinc sodium uranyl acetate salts in 95 per cent alcohol during the washing process.—To make this study approximately 0.1 g samples of the magnesium or zinc sodium uranyl acetate salts were weighed into tared Sela crucibles and the salts were rinsed with five 5-ml quantities of either (a) 95 per cent alcohol saturated with the respective sodium salt, or (b) 95 per cent alcohol containing no salt. Determinations were made at 25° and 35°C. and the data obtained are shown in Table 2. These data indicate that (a) in the case of magnesium sodium uranyl acetate both the saturated and unsaturated alcohol wash solution effect a loss of approximately 2 mg of salt per 25 ml at 25°C., (b) approximately twice this much, *i.e.*, 4 mg of salt is dissolved at 35°C. using the unsaturated alcohol wash solution, (c) the zinc sodium uranyl acetate is slightly more soluble in the unsaturated alcohol than in the saturated alcohol wash solution, and (d) the zinc salt is approximately twice as soluble in general as the magnesium salt.

TABLE 2.—*Solubility of magnesium and zinc sodium uranyl acetate salts in alcohol wash solutions*

SODIUM URANYL SALT	Mg ¹ PER 25 ml		
	25°C.		35°C.
	SATURATED	UNSATURATED	UNSATURATED
Magnesium	2.0	2.1	4.1
Zinc	3.7	5.3	8.8

¹ Average of two or more determinations.

Effect of washing all glassware with dilute nitric acid immediately before use.—Table 3 shows the values of the blanks obtained in the magnesium

TABLE 3.—*Blanks obtained with and without cleansing of all glassware with nitric acid just before use*

Mg OF PRECIPITATE	
HNO ₃ CLEANED	NOT HNO ₃ CLEANED
2.5	24.6
3.9	5.5
6.6	10.2
6.2	10.4
2.7	32.6
5.4	21.8

uranyl acetate procedure where the usual clean glassware and crucibles were used vs. where all surfaces were rinsed with dilute nitric acid and distilled water just previous to use.

Comparison of the magnesium and zinc uranyl acetate methods for the analysis of sodium in c.p. NaCl solutions.—Table 4 shows data obtained when sodium in c.p. solutions of sodium chloride was determined by the two methods. The data indicate that reasonably satisfactory analysis of known sodium solutions may be made by either method.

TABLE 4.—*Results by the magnesium and zinc uranyl acetate methods with known amounts of sodium*

Mg Na PRESENT	Mg Na DETERMINED			
	MAGNESIUM METHOD		ZINC METHOD	
0.235	0.260	0.390	0.360	0.280
0.705	0.790	0.760	0.750	0.770
1.000	1.00, 0.95	1.00	—	—
1.175	1.250	1.270	1.260	1.210
2.350	2.48	2.44	2.37	2.38
23.500	23.35	23.20	23.52	23.38

Comparison of the magnesium and zinc uranyl acetate methods in the analysis of plant samples for sodium.—Plant samples of rape, sugar beet roots, and sugar beet tops were analyzed for sodium by both methods. Table 5 shows the data obtained. Excellent duplication of values were obtained by both methods and both methods gave essentially the same results.

Precision of the results obtained by the magnesium uranyl acetate method for sodium in plants when different weights of samples were used.—Table 6 shows the precision of the results obtained by the tentative A.O.A.C. method (5) when applied to the analysis of celery and corn using samples of different weights. These results should be considered satisfactory.

TABLE 5.—*Comparison of the magnesium and zinc uranyl acetate methods in the analysis of plant samples*

PLANT SAMPLE	SAMPLE NO.	% SODIUM DETERMINED					
		MAGNESIUM URANYL ACETATE DUPLICATE DETERMINATIONS			ZINC URANYL ACETATE DUPLICATE DETERMINATIONS		
		1	2	AVE.	1	2	AVE.
Rape	1	0.375	0.369	0.372	0.337	0.401	0.369
	2	1.06	1.07	1.07	1.12	1.08	1.10
Sugar beet roots	1	0.176	0.169	0.173	0.169	0.171	0.170
	2	0.061	0.060	0.061	0.057	0.057	0.057
Sugar beet tops	1	1.08	1.06	1.07	1.06	1.06	1.06
	2	0.185	0.185	0.185	0.175	0.166	0.171

TABLE 6.—*Precision of results obtained by the tentative A.O.A.C. method for sodium in plants when different weights of samples were used*

SAMPLE	WEIGHT	SODIUM DETERMINED	
	g	mg	per cent
Celery	0.5	4.95	0.99
	1.0	9.54	0.95
	1.0	9.51	0.95
	2.0	19.93	1.00
	2.0	19.51	0.98
Corn	1.0	3.34	0.27
	1.0	2.90	0.28
	3.0	8.09	0.27
	3.0	7.66	0.26

TABLE 7.—*Analysis of plant materials with and without added sodium by the tentative A.O.A.C. method*

PLANT MATERIAL ¹	Mg SODIUM			
	PRESENT ²	ADDED	TOTAL	DETERMINED
Alfalfa	0.40	1.00	1.40	1.45
Soybean oil meal	0.12	1.00	1.12	1.27
Soybean oil meal	0.12	2.00	2.12	2.11
Oats	0.40	1.00	1.40	1.41
Corn	2.69	1.00	3.69	3.71
Celery	9.53	2.00	11.53	11.50
Celery	9.53	3.00	12.53	12.83

¹ One gram samples used.² Averages of results of duplicate determinations.

Application of the magnesium uranyl acetate method to the analysis of sodium in plant materials with and without added sodium.—Table 7 shows the results obtained when the tentative A.O.A.C. procedure (5) was applied to the determination of sodium in various plant materials with and without added sodium.

DISCUSSION AND CONCLUSIONS

The comparisons made in this investigation are not conclusive as to whether the magnesium or the zinc uranyl acetate procedures have any marked advantages over one another. The magnesium reagent shows slight advantage in that the sodium salt has less solubility in the precipitation and wash reagents. These differences are not great and apparently are taken care of in the blank values obtained since both methods gave essentially the same results when applied to the analysis of plant materials. The solubility data obtained in this investigation indicate that the precipitation of the sodium should be made at a temperature near that at which the precipitating reagent was prepared, since at higher temperatures, *i.e.*, at 35°C. it is capable of dissolving appreciable quantities of the sodium salt, and at very low temperatures, *i.e.*, 4°C., appreciable amounts of non-sodium uranyl salts may precipitate out.

The prevalence of sodium salts about the laboratory makes it necessary to exercise extreme care in the cleansing of all glass surfaces before use in the sodium procedures. Washing all surfaces with dilute nitric acid just before use resulted in uniformly low blank values.

The accuracy of both the magnesium and zinc uranyl acetate procedures was demonstrated to be satisfactory on known c.p. sodium chloride solutions in the range of 1 mg. or more of sodium. Caley (8) reported that the magnesium uranyl acetate reagent would not give satisfactory results with samples containing less than 0.2 mg. of sodium. The precision of the magnesium uranyl acetate method was shown to be satisfactory when varying weights of celery and corn samples were analyzed. However, it was found that some plant materials, such as alfalfa leaf meal, soybean oil meal, and oats, gave colloidal-type precipitates during the sodium precipitation if too large samples were taken for analysis. This may have been due to some interfering ion such as phosphorus being present in greater concentration than the method could tolerate.

Caley and Foulk (1), using magnesium uranyl acetate, found that when 10 or 20 mg of sodium were precipitated in either 100 or 200 ml of reagent, 250 mg of potassium did not interfere. Broadfoot and Browning (7) reported that with the zinc uranyl acetate method, 50 mg of potassium, 1.0 mg of sodium, and 10 ml of reagent, the results were about 10 per cent high. They found that by dissolving the precipitate in 2 ml of water and again precipitating the sodium in the usual manner theoretical recovery was obtained. As plants commonly contain as much as 40 mg of potassium

per gram and some may contain as much as 80 mg per gram (9) a sufficiently small sample must be taken for analysis in order that it will not exceed the amount of potassium that can be tolerated, or the potassium must be removed to a tolerable level before the final sodium precipitation is made.

Phosphorus, which forms uranyl phosphate, has been found to be removed satisfactorily by use of calcium chloride and ammonium hydroxide (7). Calcium hydroxide has been used to advantage in the removal of interfering phosphates (7, 10). Caley and Sickmann (11) found that 1 mg of sodium in the presence of 500 mg of aluminum or chromium may be determined within ± 0.2 mg error. They also reported that a concentration of ammonium or sulfuric ions, as can ordinarily occur in 5 ml of test solution, will not result in the precipitation of the salt complex, $(\text{NH}_4)_2\text{SO}_4 \cdot \text{MgSO}_4 \cdot 6\text{H}_2\text{O}$, where 100 ml of reagent is used. Lithium has been found to interfere markedly in the determination of sodium (2). However, lithium has been reported to occur in extremely low concentrations (12) relative to sodium in plants.

The recoveries of the small amounts of added sodium to the various plant materials should be considered satisfactory.

RECOMMENDATIONS*

The Associate Referee recommends that the magnesium uranyl acetate method should be kept tentative and that the study be continued, especially in respect to tolerance of the procedure to various levels of interfering ions such as occur in plant materials.

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REPORT ON LIGNIN AND CELLULOSE IN PLANTS

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The estimation of the nutritional value of forages and other feed stuffs has, for many years, been based on their content of ether extract, crude protein ($N \times 6.25$), ash, crude fiber, and nitrogen-free extract. The need for revision of this method has long been recognized.¹ The main basis for the Henneberg method is that crude fiber is designed as a measure of the fibrous, relatively poorly digested part of plants, while the nitrogen-free extract is designed to represent the more readily digested fraction. Crampton and Maynard² pointed out that crude fiber is often as completely digested by ruminants as is the nitrogen-free extract, thus raising a serious objection to this scheme. Norman³ showed that lignin, which is relatively indigestible, is present in varying amounts in both the crude fiber and nitrogen-free extract fractions, thus indicating further the inadequacy of the present method. Mitchell⁴ has stated the situation satisfactorily, in a review dealing with methods for evaluating feeds, when he stated "... it would seem more profitable to study the digestibility of nutrients, or classes of nutrients, better characterized and more accurately determinable chemically than those involved in the old routine method of analysis. Such additional measurements might well include lignin, cellulose, fatty acids . . . "

Crampton and Maynard⁵ have proposed that the carbohydrate fraction be defined in terms of lignin, cellulose, and "other carbohydrates" (obtained by difference) rather than in terms of crude fiber and nitrogen-free extract. Studies made with animals along these lines indicate that this proposal is a valuable one. Further study is needed and there is a real need for some agreement as to suitable procedures for lignin and cellulose. The adoption of a uniform method for lignin would be of value if for no other reason than that data from several laboratories could be used in determining the correlation between lignin content and the digestibility of various forages by livestock.

While cellulose is a reasonably well defined entity, lignin is not, and many annoyances arise when attempting a quantitative estimation. This problem has recently been discussed by Phillips.⁶ The two most widely employed methods for lignin are the 72 per cent sulfuric and the fuming hydrochloric acid ones. A thorough study of the latter procedure has been made by Phillips and coworkers and has been adopted as a tentative

¹ *This Journal*, 23, 102, (1940).

² *J. Nutrition*, 15, 383, (1938).

³ *J. Agr. Research*, 25, 529, (1935).

⁴ *J. Animal Sci.*, 1, 159, (1942).

⁵ *J. Nutrition*, 15, 383, (1938).

⁶ *This Journal*, 23, 108, (1940).

method by this Association. It is not the purpose of the present study to replace this method, but to initiate work leading to another procedure more adaptable to routine use. Since there is no absolute chemical criterion by which to judge the validity of a lignin method, this will have to be judged finally by its utility in biological studies. As a prior condition, such a method should meet an acceptable standard of reproducibility and convenience. As a starting point, a 72 per cent sulfuric acid method⁷ which already has been found of value in nutrition work has been chosen for this study. The objective in this study is to determine the variation in results as obtained by different analysts on a common sample.

A modified Norman-Jenkins method⁸ for cellulose was chosen as a starting point primarily because the celluloses are included along with the "true" cellulose $[(C_6H_{10}O_5)_n]$ in this method and Ferguson⁹ has presented evidence that the celluloses are digested to the same extent by sheep as is "true" cellulose. This method has the additional advantage that a larger proportion of the carbohydrate fraction of plants is included, particularly in the case of the grasses.

COLLABORATIVE WORK

SAMPLES USED

No. 1 (a mature Timothy hay) ground in a Wiley mill to pass a 40 mesh sieve.

No. 2 (an immature Sudan grass) obtained already ground from the Cerophyl Co., Kansas City, Missouri, and all passed a 40 mesh sieve.

No. 3 (sheep feces) ground in a Wiley mill to pass a 40 mesh sieve.

Samples 1 and 2 were submitted to the 8 collaborating laboratories with the request that 6 analyses be made. Sample 3 was submitted to 5 of these laboratories.

METHODS OF ANALYSIS

Lignin.—Extract 1 g of sample with an alcohol-benzene mixture (1:2 by volume) for 4 hours in Soxhlet or comparable apparatus (the extraction vessel may be either a coarse porosity alundum or a paper thimble, closed at the top with filter paper or plug of cotton). Wash the sample in the thimble, with the aid of suction, using two small portions of alcohol followed by two small portions of ether. Heat at 45°C. in a non-sparking oven to drive off the ether and transfer the sample to a 250 ml, wide-mouthed Erlenmeyer flask. Add 40 ml of 1% pepsin (U.S.P. grade) in 0.1 N HCl, wetting the sample well by adding a small portion of the soln, stirring or shaking thoroly, and finally washing down the sides of the flask with the remainder of the soln. Incubate at 40°C. overnight. Add 20–30 ml of hot H₂O and filter using a filter stick.¹⁰ Repeat this washing twice and then wash the residue into the flask by forcing 7–8 ml of 5% H₂SO₄ soln (by weight) downward thru the filter stick with the aid of

⁷ *J. Animal Sci.*, **5**, 285 (1946).

⁸ *J. Animal Sci.*, **5**, 306 (1946).

⁹ *Biochem. J.*, **36**, 786, (1942).

¹⁰ The filter sticks are of the type made with a pyrex fritted glass disk 30 mm. diameter, medium porosity. A thin layer of pre-ashed diatomaceous earth (hyflo supercel, or similar filter aid) is sucked onto the filter disk from a water suspension. This is usually sufficient for easy filtration; if not, add extra supercel to the material being filtered. In the author's laboratory, about one-third of the sticks purchased were found to filter slowly with some samples. It may be advisable, therefore, to purchase more than needed and discard the slow-filtering ones. It is convenient to arrange the filter sticks in a set of twelve attached to a vacuum manifold by rubber tubing.

air pressure. Wash the stick further with the H_2SO_4 soln, finally adding enough to the flask to bring the total volume to ca 150 ml. Reflux vigorously on a hot plate for one hour, adding H_2O occasionally to maintain the original volume. Filter off the acid. Wash the residue three times with 20–30 ml portions of hot H_2O , twice with 15–20 ml portions of alcohol, and twice with 15 ml portions of ether. Leave the vacuum on a few minutes to dry the residue, and transfer from the stick into the flask by tapping and brushing. Heat to drive off any residual ether. If the disk formed upon drying is difficult to break up into a finely divided state, as it sometimes is in the case of immature plant samples, disperse the residue in ether in the flask and then boil off the ether on a steam bath. Add 20 ml of 72% H_2SO_4 (by weight) at 20°C . to the residue and hold at 20°C . for two hours with occasional stirring. Add 125 ml of H_2O , filter, wash once with 20 ml of hot H_2O and again filter. Wash the residue from the filter stick and reflux as before for two hours, using 150 ml of 3% H_2SO_4 (by weight). Filter the residue on a Gooch crucible with an asbestos pad and wash with hot H_2O until free of acid. Dry at $105\text{--}110^\circ\text{C}$., and determine lignin by the loss of weight on ignition at 600°C .

Cellulose.—Extract 1 g of the sample with an alcohol-benzene mixture (1:2 by volume) in a soxhlet apparatus for 3–4 hours, or alternatively, add the solvent to the sample in a 100 ml beaker (tall form), allow to stand at room temp. for one hour with occasional stirring. Filter using a filter stick (see footnote under lignin method). Repeat with two more portions of solvent. To the extracted sample in a 100 ml beaker add 50 ml of 3% Na_2SO_3 soln and bring to a boil. Filter and add 10 ml of 0.25 *N* NaOH in dilute alcohol (3 alcohol to 2 H_2O) washing out the filter stick with this soln. Stir and neutralize to a pH of 7–9 with 1 ml of 2.5 *N* H_2SO_4 , filter, wash the residue once with 25 ml of hot H_2O , and again filter. Add 43 ml of cold H_2O and 7 ml of NaClO soln (5.25% available chlorine).¹¹ Allow to stand for 10 min., stirring occasionally with the filter stick. Filter, add 50 ml of 3% Na_2SO_3 soln and boil for at least 5 min., the filter stick serving to decrease bumping. Filter and repeat the 0.25 *N* alcoholic NaOH treatment as before. Suspend the neutralized and washed material in 50 ml of H_2O , add 1.5 ml of NaClO soln and 1 ml of 20% H_2SO_4 (by weight). Chlorine is evolved and the material frequently turns yellow. After standing for 10 min. with occasional stirring and protected from direct sunlight, filter and treat with the Na_2SO_3 soln as before. The intense purple coloration noted indicates the presence of lignin. Continue the alcoholic NaOH washings and the acid hypochlorite treatments as long as a positive reaction for lignin is found upon addition of the sulfite. After the final boiling with sulfite, filter and wash with 50 ml of hot H_2O and transfer the cellulose to an alundum crucible. Wash several times more with hot water. Dry at $105\text{--}110^\circ\text{C}$. and determine the cellulose by the weight loss on ignition.

RESULTS

The results are given in Table 1. A moisture determination in duplicate was made in each laboratory and this value used to calculate the results on a dry basis weight. In addition to the mean value, the standard error of the mean and the extremes for the six determinations are given as an indication of the variability of the results in each laboratory.

It is apparent that a greater magnitude of difference occurs between laboratories as compared to the differences within a single laboratory,

¹¹ "Chlorox" sold as a household bleach is satisfactory. This will deteriorate on long standing, so the supply should be fresh.

particularly with the cellulose values. These differences could be due to a non-uniformity of the samples, but in view of the close agreement found for the lignin values with sample No. 1, this is probably not the case. In comparing the lignin values, the results from laboratory G indicate that the temperature during the 72 per cent sulfuric acid treatment is an important factor. Omitting the values from laboratory G and H, the only

TABLE 1.—*The lignin and cellulose values for reference samples*

LAB.	LIGNIN (PER CENT DRY MATTER BASIS)		
	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3
A	10.17 ± .075 (10.0–10.3)	5.65 ± .065 (5.39–5.87)	23.48 ± .101 (23.1–23.7)
B	10.27 ± .081 (10.1–10.5)	5.40 ± .120 (5.10–5.72)	
C	10.19 ± .063 (10.0–10.4)	5.38 ± .036 (5.27–5.46)	
D	10.03 ± .021 (9.95–10.1)	5.22 ± .037 (5.10–5.35)	23.10 ± .102 (22.8–23.4)
E	10.31 ± .068 (10.2–10.5)	5.83 ± .038 (5.71–5.96)	
F	10.20 ± .188 (9.40–10.6)	6.08 ± .252 (5.04–6.58)	24.06 ± .188 (23.7–24.5)
G ¹	12.02 ± .120 (11.8–12.4)	6.23 ± .046 (6.12–6.39)	24.05 ± .135 (23.7–24.5)
H ²	10.65	6.68	24.42

CELLULOSE (PERCENT DRY MATTER BASIS)			
A	27.32 ± .029 (27.1–27.4)	24.13 ± .053 (23.9–24.2)	37.69 ± .116 (37.2–38.0)
B	31.05 ± .064 (30.9–31.2)	26.47 ± .093 (26.2–26.6)	
D	26.01 ± .685 (25.2–26.5)	25.20 ± .217 (24.9–25.3)	35.86 ± .578 (35.5–36.7)
E	29.67 ± .174 (29.3–30.4)	23.39 ± .286 (22.6–24.5)	
F	29.27 ± .153 (28.8–30.0)	23.20 ± .551 (20.7–24.4)	36.47 ± .100 (36.2–36.9)
G	33.30 ± .374 (29.9–31.6)	28.04 ± .163 (27.6–28.5)	44.41 ± .350 (43.4–45.4)
H	28.3	22.86	37.26

¹ In this laboratory the temperature during the 72 per cent sulfuric acid treatment ranged between 30.5 and 31.5°C.

² The individual values were not obtained from this laboratory in time to allow the inclusion of further information in this report.

statistically significant difference (at the 1% level) between laboratories for the lignin values on sample No. 1 is that between D and E, this difference amounting to 3 per cent. For sample No. 2, 4 of a possible 15 differences are significant (between D and ACEF). For sample No. 3, the results from laboratory F are higher than those from either A or D the greatest difference being approximately 4 per cent.

The cellulose values show a much greater variation from laboratory to laboratory even though the range of values for the six determinations within a given laboratory may be fully as limited as for the lignin values.

Consideration of these data indicates that the lignin method is reasonably satisfactory in so far as reproducibility is concerned, although further work, particularly with immature plant samples, would be desirable.

The cellulose method is clearly unsatisfactory and work leading to a closer definition of the conditions for carrying out the isolation of cellulose should be undertaken.

RECOMMENDATIONS*

It is recommended—

(1) That collaborative work on the lignin method be continued for another year using other samples of plant material.

(2) That the cellulose method be studied to determine the causes for the variation in results that have been found.

COLLABORATORS

The Associate Referee wishes to express his appreciation of the fine cooperation shown by the collaborators in this study.

C. E. French, Pennsylvania State College, State College, Pa.

Bartley P. Cardon, University of Arizona, Tucson, Ariz.

John Lawrence, State College of Washington, Pullman, Wash.

R. M. Forbes, University of Kentucky, Lexington, Ky.

R. E. Davis and Ivan Lindahl, Bureau of Animal Industry, U.S.D.A., Beltsville, Md.

Miss Jean Lowe, U. S. Plant, Soil, & Nutrition Laboratory, Ithaca, N. Y.

John K. Loosli and Miss Cornelia Hassan, Cornell University, Ithaca, N. Y.

Lorin E. Harris, Utah State Agricultural College, Logan, Utah.

REPORT ON STARCH IN PLANTS

By CARROLL L. HOFFPAUIR (Southern Regional Research Laboratory,†
New Orleans, Louisiana), *Associate Referee*

Where a considerable amount of starch is present, as in cereal grain or tubers, there are several satisfactory methods for its determination. However, when dealing with tissues low in starch but high in accompanying non-starch polysaccharides, the difficulties are accentuated. Preliminary to collaborative work it is found essential to investigate possible methods for the determination of starch in plant materials containing low percentages.

To be generally applicable such a method should meet the two criteria stated by Denny (1), namely, the method must give correct values when pectin, non-starch polysaccharides and protein are present in the sample; and the method should give zero or trace values on samples containing little or no starch by qualitative tests, but containing high amounts of substances which might interfere. Steiner and Guthrie (2) have developed a method which separates starch from the interfering materials usually

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 45 (1949).

† One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

present in plant materials. It consists of treatment of the sample at boiling temperature with dilute ammonium carbonate solution, precipitation of the starch with iodine, decomposition of the starch iodide, reprecipitation with iodine, decomposition of the starch iodide, precipitation of the starch with alcohol, dispersion in calcium chloride, precipitation of any remaining protein with uranyl acetate and determination of the optical rotation. It is, however, recommended by the authors only for samples containing 10 per cent or more of starch since when less starch is present the optical rotation is too small to be read precisely. In an attempt to adapt the Steiner-Guthrie method to the determination of low percentages of starch, two methods of evaluating starch in the final calcium chloride dispersion were tried. The first of these was an acid hydrolysis of the starch to glucose followed by a reducing sugar determination. The second was a colorimetric method based on the blue starch-iodine complex.

SAMPLE MATERIAL

The following samples were used to test the method:

1. *Orange rind*.—Composite of many kinds of orange peel that had been dropped into boiling alcohol, ground in a food chopper, Soxhlet extracted with 80% alcohol and ground in a ball mill.
2. *Cottonseed meal*.—Composite of ground cottonseed kernels thoroughly extracted with ethyl ether, and then ground in a ball mill.
3. *Jerusalem artichokes*.—Artichokes were sliced and dropped into boiling alcohol, then ground in a Waring Blendor, dried and ground in a ball mill.
4. *Cotton root bark No. 1*.—Bark was extracted with alcohol and ground in a ball mill.
5. *Cotton root bark No. 2*.—Another sample of bark prepared as above.
6. *Cotton leaves*.—Leaves extracted with alcohol and ground in ball mill.

METHODS

Acid hydrolysis procedure.—Pipet a suitable aliquot (containing ca 10 mg of starch) of the calcium chloride dispersion obtained by the Steiner-Guthrie method into a 125-ml ground-glass stoppered Florence flask. Add water to make volume 50 ml and then add 5 ml of hydrochloric acid, sp. g. 1.125. Attach the flask to a reflux condenser and boil on a hot plate for 2.5 hours.

After cooling, neutralize the soln to the phenol red end point (pH 7 to 8) with 20% sodium hydroxide soln. Transfer to a 100-ml volumetric flask, make to volume with water, and mix well. Place a portion of the soln in an Erlenmeyer flask, add dry sodium oxalate and shake for about an hour. Test a drop of the supernatant for calcium on a black spot plate with sodium hydroxide soln. Continue the addition of sodium oxalate followed by shaking until the calcium has been removed. Centrifuge the mixture and analyze 5 ml of the supernatant for reducing sugars by the Somogyi modification of the Shaffer-Hartman method (3). Multiply the value of glucose found by the factor 0.93 to convert to starch.

Colorimetric procedure.—Pipet an aliquot (containing ca 10 mg of starch), of the calcium chloride dispersion obtained by the Steiner-Guthrie method, into a 100-ml volumetric flask. Dilute to volume with water. Mix well. Pipet a 25-ml aliquot of the diluted soln into a 50-ml volumetric flask. Dilute to ca 40 ml with water. Add 2.5 ml of 10% acetic acid and 1 ml of 5% potassium iodide soln. Mix by swirling. Pipet 5

ml of 0.01 *N* potassium iodate soln into the flask. Dilute to volume and mix thoroly. Read the transmission of the soln in a photoelectric colorimeter, using a filter with maximum transmission at 565 $m\mu$ after first setting the instrument at 100% transmission with a reagent blank. Determine mg of starch in the sample aliquot by means of a standard curve obtained from a known starch soln.

DISCUSSION

In Table 1 are shown the values obtained by the methods described together with values obtained by other well known methods on the samples described and used (4, 5).

TABLE 1.—*Comparison of methods for determination of starch*

METHOD	STARCH FOUND ¹					
	COTTON ROOT BARK NO. 1	COTTON ROOT BARK NO. 2	COTTON LEAVES	ORANGE RIND	COTTONSEED MEAL	JERUSALEM ARTICHOKEs
	per cent	per cent	per cent	per cent	per cent	per cent
Values obtained on Steiner-Guthrie dispersion						
Acid hydrolysis	4.6	2.7	0.9	1.7 1.7	1.1	0.4
	5.3	3.6	1.2	1.3 1.4	1.1	0.3
Colorimetric (potato starch standard)	4.6	6.0	2.0	0.4 ²	0.2 ²	0.0
	4.6	6.0	2.0			0.0
Colorimetric (isolated starch standard)	4.1	5.4	1.8			
	4.1	5.4	1.9			
Values obtained by other methods						
Taka-diastrase	4.9 ³	6.0 ³	1.7 ³			
	5.1 ³	6.2 ³	1.8 ³			
Malt-diastrase				12.8 ³	9.8 ³	27.4 ³
Hopkins				22.6 ³	-4.4 ³	-18.0 ³
Steiner-Guthrie	3.1	4.6	0.9	-0.2 ³	-0.6 ³	-0.3 ³

¹ All values were obtained on the samples used and described.

² Values obtained by Steiner and Guthrie (2).

³ Values obtained by Eaton and Ergle (9).

Qualitative tests with iodine indicated that the first cotton root bark sample contained somewhat less starch than the second sample and that the cotton leaves contained less than the bark samples. All three of these

samples were deeply colored. Nielson (6, 7) has pointed out that the intensity of the starch-iodine color is dependent on the amylose-amylopectin ratio. Consequently the starch dispersions from samples of cotton root bark and cotton leaves were compared to color standards made from starch isolated from the respective samples as well as with those from white potato starch. Because of the difficulty of isolating pure starch from such materials the values so obtained are probably somewhat high. They are, however, slightly lower than those obtained using potato starch as a standard. The starch contents were considerably below the best range of the Steiner-Guthrie method, and the values obtained by it are lower than the colorimetric values while those by the taka-diastrase method are slightly higher for the bark samples and in good agreement for the sample of leaves. With each of these methods the starch contents were in the same order as indicated by the qualitative iodine test, and the agreement between duplicate determinations was satisfactory. The acid hydrolysis method on the other hand gave poorly agreeing duplicate values and reversed the order of the starch contents of the cotton root bark samples.

Qualitative test indicated that the orange rind sample contained very little starch. The colorimetric value was in agreement with the qualitative test. The Steiner-Guthrie method gave a low negative value and the acid hydrolysis method a somewhat higher value than the colorimetric method. Both the malt-diastrase and Hopkins methods gave values which were absurdly high. These high values are no doubt due to the interference of polysaccharides such as pectin. A similar pattern of results was obtained with cottonseed meal, which qualitative test indicated contained only a trace of starch. In this case the interference of protein gave negative values with both the Hopkins and the Steiner-Guthrie methods.

The sample of Jerusalem artichokes was starch free by qualitative test. The values obtained again emphasize the interference of non-starch polysaccharides. No starch was found with the colorimetric procedure.

While none of these methods are satisfactory for samples low in starch it is believed that the measurement of the light absorbed by the starch iodide complex shows considerable promise. Recent investigations of Swanson (8) suggest the possibility that the difficulties caused by the varying amylose-amylopectin ratio in starches from different natural sources may be overcome by suitable spectrophotometric measurements. It is recognized that the scattering of light by the dispersed starch may be an important factor in such measurements.

RECOMMENDATION*

It is recommended that the spectrophotometric measurement of starch-iodine dispersions be investigated as a possible method for the determination of starch in samples low in starch.

* For report of Subcommittee A, and action of the Association, see *This Journal*, 32, 45 (1949).

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REPORT ON BORON IN PLANTS

By CALVIN M. AUSTIN and L. K. WOOD (*Associate Referee*), Kentucky
Agricultural Experiment Station, Lexington, Ky.

Because of personnel changes and other difficulties work on the boron problem was delayed, and results obtained this year are not felt to be appropriate for a report to the A.O.A.C.

At the present time work is underway to further investigate the "Chromotrope B" method of Austin and McHargue,¹ particularly ignition technique. The application of the method to soils and other biological material is also being investigated.

It is requested that those persons interested in collaborating in this work write the Associate Referee. It is hoped that several will respond so that an adequate collaborative program can be set up and a report submitted next year.

RECOMMENDATIONS*

It is recommended that the "Chromotrope B" method for boron be further investigated and that collaborative work be undertaken this year.

No report was given on sampling (plants) sugar, copper and cobalt, carotene, or pectin.

No report was given on spectrographic methods.

¹ Calvin M. Austin and J. S. McHargue *This Journal*, **31**, 427 (1948).

* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 45 (1949).

MONDAY—AFTERNOON SESSION

REPORT ON PROCESSED VEGETABLE PRODUCTS

By V. B. BONNEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

It is recommended—*

(1) That studies of methods for determining quality factors in canned and frozen fruits and vegetables be continued.

(2) That studies of methods for the estimation of the enzymatic activity of frozen fruits and vegetables be continued.

(3) That studies of the determination of moisture in dried vegetables be continued.

REPORT ON PEROXIDASE IN FROZEN VEGETABLES

By M. A. JOSLYN (Food Technology Division, University of California, Berkeley 4, Calif.), *Associate Referee*

A variety of methods have been reported in the literature for both the quantitative and the qualitative measurement of peroxidase activity, and several of these methods have been applied to the determination of peroxidase activity in frozen vegetables with a considerable amount of conflicting data (see review by Joslyn (1)). There has been no critical investigation of the determination of peroxidase since that of Balls and Hale (2) in 1933. Their procedure was subjected to collaborative study chiefly for cereal products, but was applied in modified form by Joslyn *et al* (3) to the determination of peroxidase activity in frozen vegetables. The methods that have been proposed for the determination of plant peroxidase fall into the following categories:

(1) *Purpurogallin procedures.* The quantity of purpurogallin formed by oxidation of pyrogallol by peroxidase in presence of hydrogen peroxide has been repeatedly shown by Willstätter and his coworkers (4) to be directly proportional to the time of reaction and to the quantity of peroxidase present. The purpurogallin formed may be determined gravimetrically or colorimetrically after several extractions with ether. While this procedure has the advantage that it is not affected by the presence of catalase, it is time consuming, not very sensitive, nor sufficiently specific. Pyrogallol under the conditions used (5.0 grams of pyrogallol, 50 mg of hydrogen peroxide in reacting volume of 2 liters without buffer at 20°C; usual reaction time of 5 minutes) is not oxidized specifically by peroxidase as polyphenol oxidase and certain catalytically active metals may interfere.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 51 (1949).

(2) *Titrimetric hydrogen peroxide procedures.* Balls and Hale (2) introduced the iodometric determination of residual hydrogen peroxide as a simple and rapid method of measuring peroxidase activity. In their procedure the quantity of peroxide decomposed in the presence of 0.02M phosphate buffer at pH 8, 0.0016 N hydrogen peroxide, and 0.625 gm of pyrogallol in a reaction mixture of 250 ml, is determined periodically by titrating iodine liberated from 25 ml aliquots after reaction with potassium iodide in acid-pyrogallol solutions with standard thiosulfate (5). The reaction is allowed to proceed in a glass stoppered cylinder at 30°C. Freshly boiled water is used as a diluent, covered with air-free paraffin oil, and stirred with hydrogen or nitrogen just prior to introduction of enzyme solution. They found the decomposition of peroxide to be linear with time, provided the peroxide concentration is low (below 0.002 N), and the amount of enzyme is such that not more than one-fourth to one-third of the peroxide is decomposed.

Essentially similar conditions were used in our tests (3), except that paraffin oil was not used and the air in the reaction vessel was not swept out with nitrogen. The end point in the titration, however, was found to be sharper if autoxidation of the pyrogallol did not occur. This was best controlled by vacuumization of the reaction mixture followed by release of vacuum with nitrogen. Difficulty was experienced in obtaining constant P.E. values with the more active samples, even when only one-fourth of the peroxide present was decomposed in 10 minutes. The best results were obtained when the volume of 0.01 N thiosulfate used for the titration of the aliquots decreased from about 4.5 ml to 3.9 ml in the course of about 10 minutes.

Morris *et al* (6) further modified the Balls and Hale (2) titrimetric procedure by substituting guaiacol for pyrogallol, using a lower pH (5.6) because of change of substrate and the addition of an excess of standard thiosulfate to the mixture in which the unused hydrogen peroxide liberated iodine from potassium iodide. The latter change made it possible to titrate with standard iodine to a sharp end point, even on extracts containing considerable soluble starch.

(3) *Colorimetric procedures.* A large number of indicators, chiefly mono and polyhydroxy benzenes, mono and di-amines, amino phenols and related compounds, have been introduced as reagents for peroxidase. In the early investigations, the activity of peroxidases was investigated almost exclusively with these chromogenic substances. The rate of pigmentation during enzymatic reaction, however, is not necessarily proportional to the rate of oxidation, for pigment development is markedly influenced as to degree and color by pH, presence of reducing agents such as ascorbic acid which may retard color development for some time, and other factors.

In recent years, guaiacol (7), p-amino benzoic acid (8), α naphthol and

the Nadi reagent (9) have been used for quantitative assay. Of these guaiacol is more specific for plant peroxidase, since it is not oxidized by the usual plant phenolases and lends itself well to photo-electric-colorimetric measurement of rate of formation of the orange-brown oxidation product. In the procedure developed by Masure and Campbell, a 2 ml aliquot of the filtrate from a pH 4.5 citrate buffer extract of vegetable tissue is added to 20 ml of water in a test tube, followed by 1 ml of 0.5 per cent guaiacol in 50 per cent alcohol and 1 ml of 0.085 per cent hydrogen peroxide. The contents of the tube are then mixed, a portion of the reaction mixture transferred into 14 mm Klett-Summerson colorimeter tube, and placed in the colorimeter using the 420 millimicron filter. From the galvanometer readings taken at several time intervals, the divisions of color per minute are calculated and used as an index of peroxidase activity of extract.

In the Morris, *et al* (6) modification of this procedure, the reaction mixture consisted of 2.5 ml of 1 *M* acetate buffer of pH 5.6, 1 ml of 10 per cent guaiacol in 95 per cent alcohol, and sufficient water to make 50 ml, when the enzyme extract (usually 1–5 ml depending on activity) and 1 ml of 0.75 per cent hydrogen peroxide solution were added. The completed mixture was immediately stirred and a portion poured into the Klett-Summerson colorimeter tube. The rate of color formation, which was linear at first, was measured, using filter No. 42 (400–465 m μ). Since the pH optimum for the oxidation of guaiacol is close to 5.6, this change in pH used for measurement is desirable.

(4) *Direct oxidation of iodide.* It has long been known that peroxidase will catalyze the direct oxidation of dilute acid iodide solution by hydrogen peroxide, and recently the iodide oxidation has been proposed as a field test for dehydrated vegetables (10).

(5) *Ascorbic acid oxidation.* Since Szent-Gyorgyi's early demonstration (11) that "hexuronic acid" retarded the rate of color formation of peroxidase reagents, it has been demonstrated that peroxidase in presence of hydrogen peroxide and a suitable phenolic compound will rapidly oxidize ascorbic acid (12). Several methods based on the volumetric or photometric determination of the rate of oxidation of added ascorbic acid have been proposed (13).

It is fairly well established that activity measured by these various methods may differ widely, particularly when different substrates are used in gravimetric, titrimetric or volumetric methods. When the same substrate is used, agreement between colorimetric methods such as that of Morris *et al* (6) and the titrimetric method is good, at least for some vegetables. Not only does the relative activity of the peroxidases of a particular vegetable tissue vary with indicator used, but the apparent rate of thermal inactivation of the vegetable peroxidases *in situ* also varies with the indicator (1). Consequently, in interpreting peroxidase activity

as a possible index of quality retention in frozen vegetables, it is necessary to select a procedure which will measure that peroxidase activity which has a thermal destruction rate similar to the enzymes involved in off-flavor formation.

In the development of an appropriate peroxidase method we must also have a measure of sampling error due not only to the natural variability in vegetables, themselves, but also due to the unequal distribution of peroxidase in various portions of the plant. As a rule peroxidase activity is greatest in those vegetable parts which are most active in respiration, growth, etc., and is largely concentrated along the vascular bundles. The presence of thermostable peroxidase-like substances in localized regions of vegetable tissue as well as of naturally occurring inhibitors are additional factors involved. Furthermore, difference in degree to which the tissue peroxidases are made available for reaction, as well as the conditions of the test, will influence the results. There are significant differences between the intensity of color produced by test reagents in cut tissues macerates of such tissues, or filtrates prepared from them. Although filtration will remove portions of tissues containing thermostable peroxidase-like substances, it will also remove peroxidase activity that may be significant in testing for adequacy of the scalding procedure.

The proper preparation of tissue extracts for analysis is an important problem. Balls and Hale (2) proposed that 5 grams of material to be analyzed be ground fine in a mortar with clean, sharp sand, and then triturated with 45 ml of 0.1 *M* phosphate buffer ($pH = 8$) added gradually. The sand and larger particles are settled or centrifuged out and a suitable portion of the supernatant liquid used in enzyme determination. Joslyn, *et al* (3) modified this procedure somewhat by first grinding a representative sample of frozen vegetables in a food chopper while still frozen, then triturating a 5 gram aliquot with 10 grams of sharp quartz sand and 45 ml of 0.1 *M* phosphate buffer of pH 8.0, allowing the mixture to stand 15 minutes, and then separating the aqueous portion from the remaining fibers by pressing through cheesecloth.

Masure and Campbell (7) placed 60–150 gm of tissue in a Waring blender jar with 3.0 ml of pH 4.5 citrate buffer per gram of tissue, saturated the mixture and displaced air in the jar with nitrogen, and then ground at full speed for 2 minutes with good flow of nitrogen. The extract was then filtered through cotton milk filter, discarding the first 10 or 20 ml of filtrate. Morris *et al* (6) prepared their extracts (of fresh carrots) by comminuting 50 gram portions of diced samples in a Waring blender for 3 minutes with about 1 gram of calcium carbonate and sufficient cold 2 per cent aqueous sodium-chloride solution to make a total volume of 200 ml. The larger solid particles were then removed by filtration through a gauze-backed cotton milk filter. They reported that the enzyme activities of filtrates prepared as above, unfiltered salt-carbonate suspensions

and aqueous suspensions of carrots were about the same. Filtrates of aqueous extractions, however, exhibited only two-thirds of the total peroxidase activity.

Although Willstätter and his collaborators (4) found that the peroxidase of horseradish was fairly firmly adsorbed upon the tissue particles and readily solubilized by dilute alkalis, there is but little data on the relative solubility of other plant peroxidases. There are indications that incomplete extraction of peroxidases from vegetables such as asparagus occurs when acid buffers are used.

In the light of the problems summarized above, the investigations carried out the past year were purely exploratory to determine the most appropriate test procedure and method of preparation of sample. It was tentatively decided to concentrate on the guaiacol procedure because previous experience has shown that guaiacol-peroxidase activity appears to parallel off-flavor formation in most of the frozen vegetables tested. It is intended, however, to compare this with the ascorbic acid and titrimetric procedures. A suitable method for sampling, preparation of the sample, and extraction of peroxidase, has not yet been developed. Since the accuracy and precision of a given method must be tested in more than one laboratory and by more than one investigator, considerable thought was given to the feasibility of collaborative assay of frozen vegetables which would have to be shipped over long distances. Arrangements are being made to do this, however, in at least four laboratories. Now that the preliminary survey has been made, better progress on this problem is possible.

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No report was given on quality factors, moisture in dried vegetables, or catalase in frozen vegetables.

REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Referee*

The Committee of the A.O.A.C. requested the Referee to continue the study of a quantitative estimation of Tartrazine (FD&C Yellow No. 5) in presence of Sunset Yellow F.C.F. (FD&C Yellow No. 6). As a result of the investigation the following method is proposed.

The method is based on the observation that Sunset Yellow F.C.F. is completely destroyed by hydrogen sulphide heated under pressure with the formation of an almost colorless solution. Tartrazine on the other hand being more resistant to this treatment is affected only to a slight extent. Since the amount of Tartrazine destroyed appears to be proportional, results of a quantitative order are possible by applying a correction factor.

METHOD

Prepare 1% soln of the dye or dye mixture. For orientation purposes place 10 ml of a 0.01% soln in a test tube and add 0.5 ml 10% sodium hydroxide soln. The presence of FD&C Yellow No. 6 manifests itself by a more or less brownish red coloration depending on the respective amounts. FD&C Yellow No. 5 is almost unaffected. Add to this alkaline color soln a few crystals at a time of sodium hydrosulphite $\text{Na}_2\text{S}_2\text{O}_4$ with constant shaking. FD&C Yellow No. 6 becomes colorless, while FD&C Yellow No. 5 remains yellow. This procedure detects less than 0.5% of FD&C Yellow No. 5 in a dye mixture. Conversely, presence of FD&C Yellow No. 6 can be detected by comparing above sodium hydroxide soln of color mixture with standard soln of FD&C Yellow No. 5 of equal strength and alkalinity. Looking thru tubes vertically against white background, FD&C Yellow No. 6 will be discernible by slight brownish coloration.

Withdraw several 20 ml portions of the 1% dye soln of above and estimate total dye by titrating with 0.1 *N* titanium trichloride using sodium citrate as buffer following procedure as outlined in 6th Ed. 21.39(a). In case of over-titration, back-titrate immediately with a standard soln of amaranth or other dye. If qualitative tests listed above gave evidence of presence of both dyes pipet that amount of dye soln which is equivalent to ca 15 ml of 0.1 *N* titanium trichloride soln into two or more pear-shaped Pyrex pressure flasks (capacity 110-150 ml). Add to each flask:

Tartaric acid	— 5.0 g.
Water	—50 ml
Alumina cream	—25 ml
1% soln of FD&C Green No. 2	—0.5 ml

Total volume should not exceed 100 ml. Introduce into flasks a steady stream of

hydrogen sulphide gas for exactly 5 min. Stopper flask securely and place in sterilizer or autoclave and heat with steam at 15 lbs. pressure for exactly one hour. When cool transfer contents with hot water into a 500 ml Erlenmeyer flask and add 5.0 g sodium bitartrate and water to make to 200 ml and titrate the hot soln slowly with the 0.1 N titanium trichloride soln until a very light greenish yellow end point is reached. Treat blank containing the tartaric acid, alumina cream, and FD&C Green No. 2 exactly as above and titrate (ca 0.08–0.10 ml). Subtract blank from above titration. Difference $\times 1.14$ (correction factor) = value for Tartrazine in the aliquot. Subtract this corrected volume from the total color titration. Difference $\times 1.013$ (buffer correction) = value for Sunset Yellow F.C.F.

This method possesses the advantage of comparative simplicity with reproducible results.

RECOMMENDATIONS*

The Referee recommends—

(1) That the method for the quantitative estimation of ED&C Yellow No. 5 (Tartrazine) in presence of FD&C Yellow No. 6 (Sunset Yellow F.C.F.) be tested collaboratively.

(2) That the rapid method of detection of small amounts of Tartrazine FD&C Yellow No. 5 as published in *Methods of Analysis*, 20.125, be made official, final action, with slight modification adopted last year.

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green S.F. Yellowish), FD&C Green No. 3 (Fast Green F.C.F.), and FD&C Blue No. 1 (Brilliant Blue F.C.F.).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow AB), FD&C Yellow No. 4 (Yellow OB), FD&C Orange No. 2 (Orange SS), and FD&C Red No. 32 (Oil Red XO).

(5) That investigational work on analytical methods for coal-tar colors certifiable for use in foods be conducted.

REPORT ON DAIRY PRODUCTS

By GUY G. FRARY (State Chemist, Vermillion, South Dakota), *Referee*

Credit is due the Associate Referees who completed work during the year and have given their reports here. I hope sincerely that before another meeting of the Association some other items may have been given sufficient study to justify making additional methods in the chapter official.

RECOMMENDATIONS†

It is recommended—

(1) That the Sanders-Sager Method for phosphatase in dairy products be further studied.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 52 (1949).

† For report of Subcommittee C and action of the Association, see *This Journal*, 32, 52 (1949).

(2) That studies be conducted to compare the Sanders-Sager Method for phosphatase in milk or cream with the methods appearing under Nos. 22.43 and 22.57.

(3) That studies on methods for the detection of chlorine in milk and for the detection of reconstituted milk be discontinued.

(4) That studies be continued on the determinations of the acidity of milk and ash in milk.

(5) That studies of methods for preparation of butter samples, including the use of mechanical shaking and cooling of samples, be continued.

(6) That work be discontinued on methods for preparation of samples of frozen desserts, and on methods for determination of fruits and other characterizing ingredients in such products.

(7) That studies of the Babcock Method for fat in milk be made and with particular attention to homogenized milk.

(8) That further study of the Roesse-Gottlieb Method for fat in dairy products be made.

(9) That the recommendation of the Associate Referee as to change of wording of the instructions for digestion of sample in 22.130, fat in cheese, be adopted.

(10) That the sour serum method for detecting added water in milk, 22.29, be dropped, first action

(11) That further study be made of the acetic serum method, 22.28, and of the copper serum method, 22.30, for added water in milk.

REPORT ON SAMPLING, FAT, AND MOISTURE IN CHEESE

By WILLIAM HORWITZ (*Associate Referee*) and LILA KNUDSEN (Food and Drug Administration, Federal Security Agency, Minneapolis 1, Minnesota and Washington 25, D. C.)

A more extensive collaborative study of the official and modified methods for fat and moisture¹ has been conducted and analyzed statistically during the past year. Portions of the same cheese used in the previous study (which had been stored in the frozen condition) were prepared in the same manner as described in the previous report.¹ The design of the experiment was extended by submitting two sets of samples to each of five laboratories with the request that each set be analyzed by a different chemist, in duplicate, if possible. With this experimental design, the total variation could be separated into (1) the variation between laboratories (how well two laboratories can check each other), (2) the variation between collaborators within a laboratory (how well two chemists in the

¹ *This Journal*, 31, 300 (1948).

same laboratory can check each other), and (3) the variation between duplicate samples run by the same collaborator (how well a single chemist can check himself). The data are also used to determine which, if any, of the two methods for fat and moisture yields more reproducible results.

All of the participating laboratories analyzed the samples for moisture in duplicate. Four of the five laboratories analyzed the samples for fat in duplicate; the fifth laboratory made single determinations for this constituent because of a temporary shortage of the required equipment. Determinations of fat and moisture in additional samples numbers 3 and 4 were made, however, by this laboratory.

METHODS

MOISTURE

I. Official Method: Sec. 22.124.

II. Modified Method: 1.25 hours in the forced draft oven at 130°C. See reference 1.

FAT

III. Modified Method: Direct weighing of sample into and digestion in the Mojonnier tube. See reference 1.

IV. Official Method: Sec. 22.130.

Fat on the dry basis is to be reported using the average moisture by the official method (I).

RESULTS AND DISCUSSION

Since a table listing over 700 results for fat, moisture, and the derived value of fat on the dry basis would not be informative, the data are presented graphically as differences from the average of each pair of samples. A general average was calculated for all results by the official A.O.A.C. moisture method on samples 1 and 2 (duplicate samples of the same cheese) and differences were obtained between each result and that general average. The same thing was done for samples 3 and 4, and then for samples 5 and 6. These differences are plotted together on the same scale and grouped by collaborator and laboratory as shown on the left-hand side of Figure 1 for the official moisture method. The right-hand side of Figure 1 shows the same thing for the modified moisture method. Figure 2 treats similarly the modified method for fat and the official method for fat, while Figure 3 does the same for fat on the dry basis.

In order to assess the significance of the differences between laboratories and between collaborators within the same laboratory and also to obtain estimates of the types of variations mentioned in the first paragraph, analyses of variance were performed on the data for each method separately. In most cases the variation between laboratories contributed a significant amount of variation. The difference between collaborators within a laboratory was also very significant ($P < .01$). Since the duplicate analyses on a single sample were made at the same time, they would be

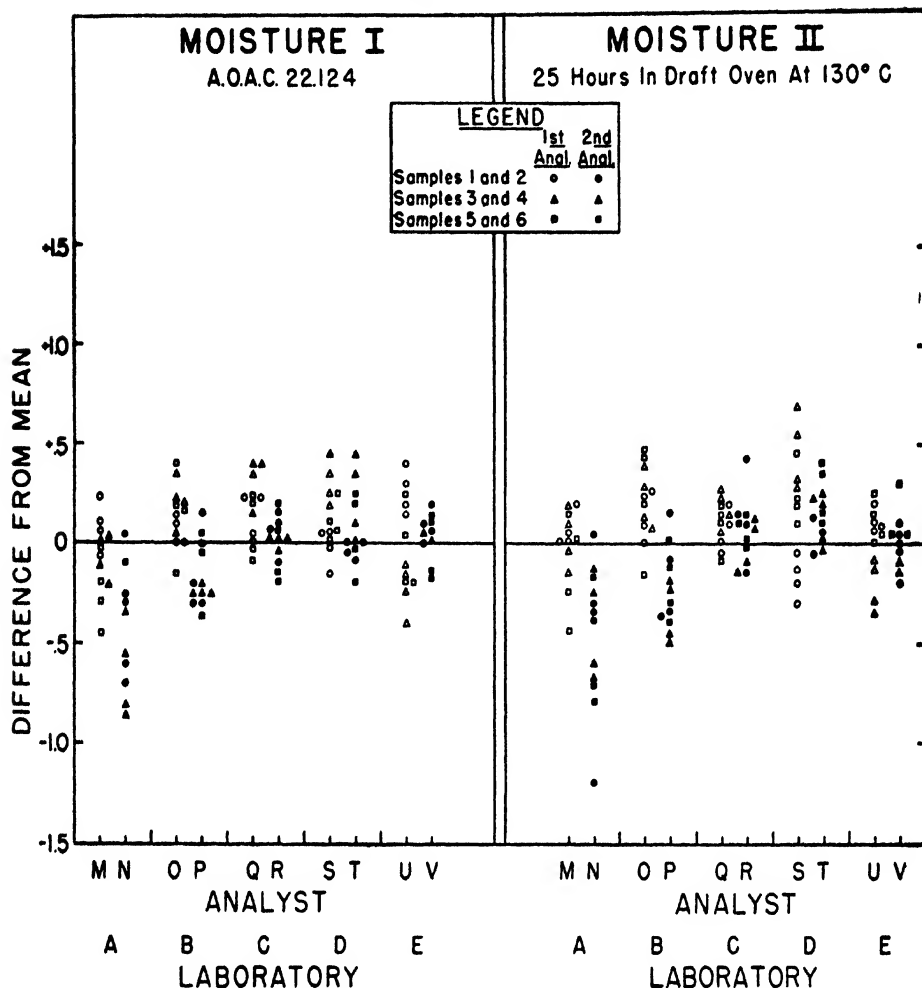


FIG. 1.—Moisture.

expected to check more closely than the analyses on the duplicate *samples* of the cheese (*e.g.*, samples 1 and 2 were duplicate samples from the same cheese mixture and duplicate analyses were made on each sample). This proved to be the case.

The three most important components of variation are given for each method in Table 1; (1) the variation between laboratories, (2) the variation between collaborators within a laboratory, and (3) the variation between duplicate samples run by the same collaborator. These variations are shown in two ways: in terms of the standard deviation and in terms of variations to be exceeded 1 time in 20 (*i.e.*, limits within which one would expect the check determinations to fall 19 times out of 20, here called the $P=.05$ limits).

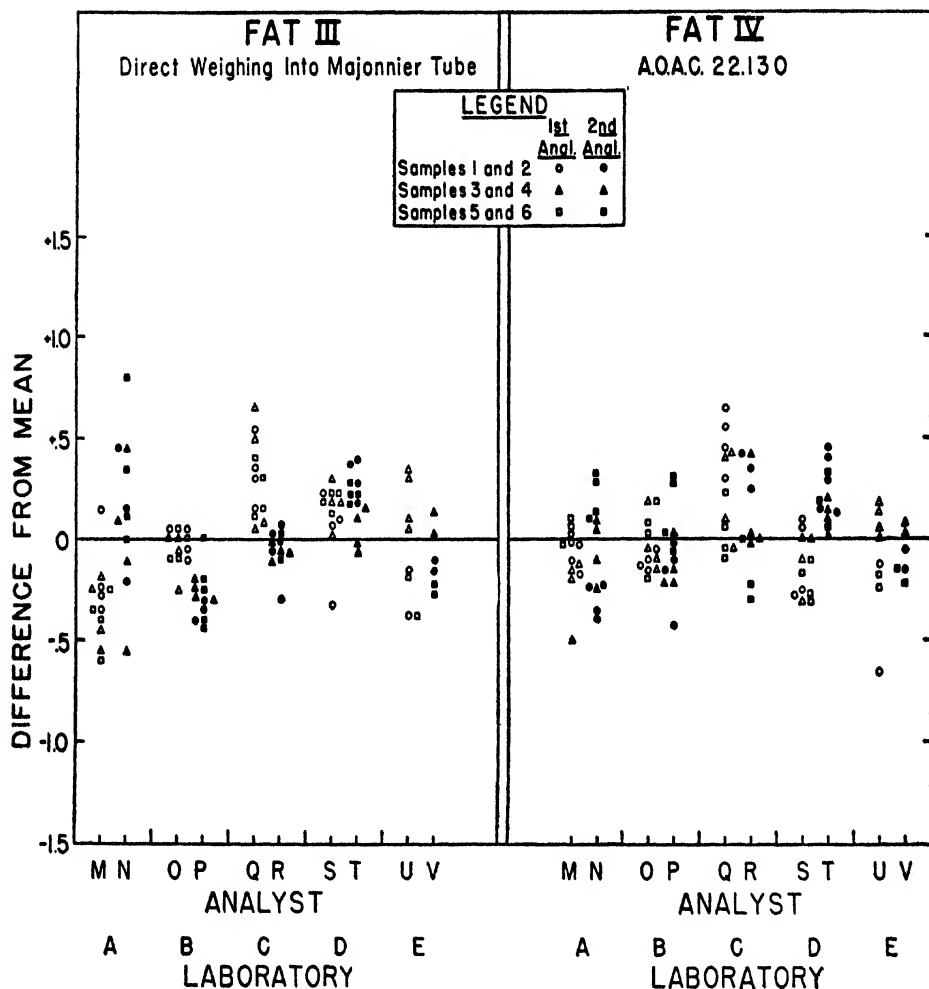


FIG. 2.—FAT.

As an explanation of the use of Table 1, the following illustration can be given. Suppose one chemist analyzes a sample of ground cheese for moisture by the official A.O.A.C. method and finds it to contain 36.50% moisture. One would expect another chemist at the same laboratory to get a result above 36.99 per cent or below 36.01 per cent only one time in 20 (36.50 ± 0.49 per cent). The same chemist should expect to obtain a result at another time on the same sample of cheese between 36.19 per cent and 36.81 per cent (or 36.50 ± 0.31 per cent)

Larger limits could be taken that could be exceeded only one time in one hundred by taking a larger multiple of the standard deviation (*i. e.*, 2.58 times the standard deviation for $P = .01$). The $P = .05$ limits in Table 1 were obtained by using 1.96 times the standard deviation.

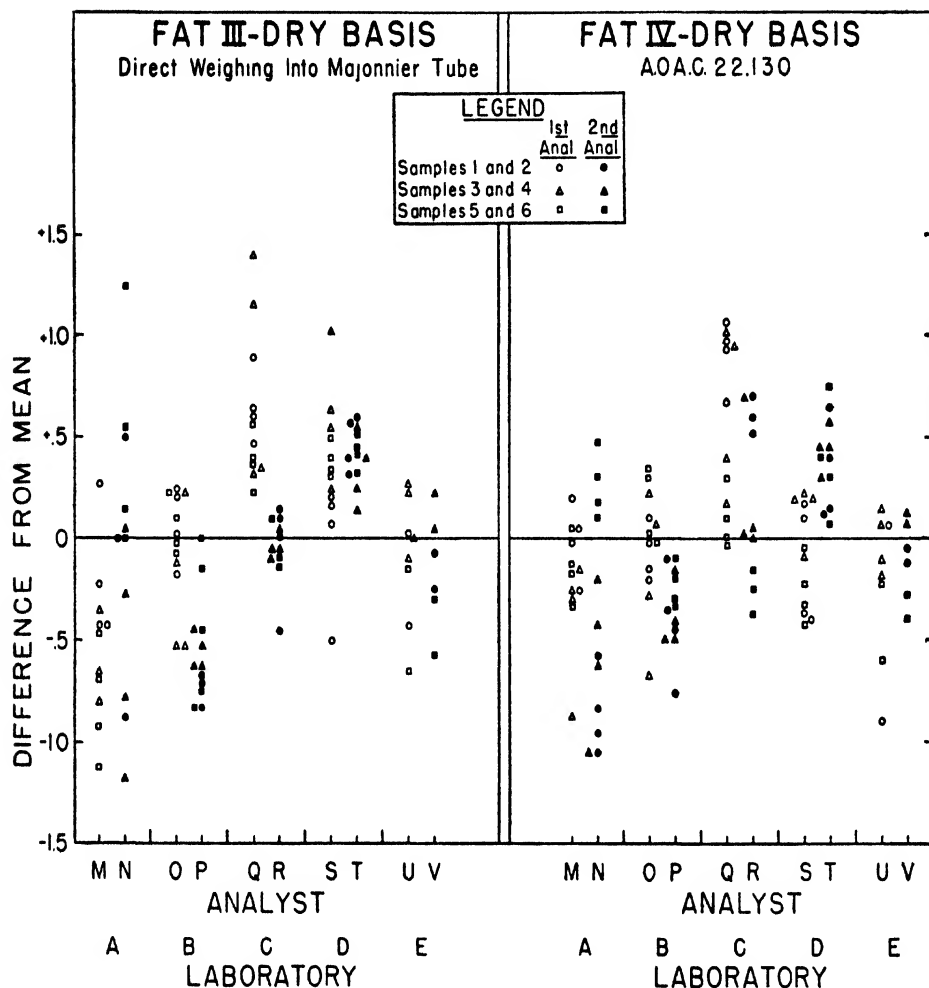


FIG. 3.—Fat—Dry Basis.

From Table 1 it can be seen that the official methods in use at present seem to be slightly more precise than the modified methods since the standard deviations are smaller.

The measures of variation obtained in this study on the moisture methods are not very different from those obtained in the previous study.¹ However, the variations in the fat methods are much smaller in this study. The standard deviations for check results between laboratories were 0.48 per cent and 0.44 per cent on the official method and the modified methods, respectively, in the previous study as compared with the respective figures of 0.24 per cent and 0.32 per cent in this study.

Since the statistical analysis indicated that the official method was somewhat greater in precision, it was not felt advisable to recommend

a change in the official method unless it could be demonstrated that a substantial improvement in technique or convenience would result from the change. A letter was sent to the collaborators requesting their opinion as to whether the modified method offered such a substantial improvement. Four of the collaborators expressed an outright preference for the official method, two preferred the modified method, one stated that he

TABLE 1.—*Components of variation*

CHECK RESULTS TO BE OBTAINED:	METHOD I		METHOD II	
	MOISTURE A.O.A.C. 22.124		MOISTURE 1.25 HOURS IN DRAFT OVEN AT 130°C	
	STANDARD DEVIATION*	P=.05 LIMITS	STANDARD DEVIATION*	P=.05 LIMITS
(1) Between laboratories	± .29	± .57	± .31	± .61
(2) Between collaborators within one laboratory	± .25	± .49	± .27	± .53
(3) By one collaborator	± .16	± .31	± .20	± .39

CHECK RESULTS TO BE OBTAINED:	METHOD III		METHOD IV	
	FAT DIRECT WEIGHING INTO MOJONNIER TUBE		FAT A.O.A.C. 22.130	
	STANDARD DEVIATION*	P=.05 LIMITS	STANDARD DEVIATION*	P=.05 LIMITS
(1) Between laboratories	± .32	± .63	± .24	± .47
(2) Between collaborators within one laboratory	± .28	± .55	± .19	± .37
(3) By one collaborator	± .19	± .37	± .15	± .29

* Obtained by using variance components or corrected variances.

saw no particular advantage of the modified procedure over the official method, and one suggested that the modified procedure be made optional for dry, granular cheese, while no statements were received from the other two collaborators. On the basis of these statements, it would appear that there is insufficient advantage to be gained by the method of direct weighing into the Mojonnier tube to warrant a recommendation to modify the official method for fat.

Several collaborators expressed the opinion that the exclusive use of sand in the official method is too restrictive and that the use of other antibumping agents should be made optional. Glass beads, porcelain chips, and carborundum grains have been employed for this purpose by analysts without noticeable effect on the results. It is therefore recom-

mended that the statement in the cheese method for fat, 22.130, "Add ca 0.5 g of sand, previously digested with HCl, to prevent bumping . . ." be changed to read "Add a few glass beads, or other inert material, previously digested with HCl, to prevent bumping, . . ."

ACKNOWLEDGMENT

Grateful acknowledgment is due the following collaborators (all of the Food and Drug Administration): John H. Bornmann and Leon E. Wener, Chicago; F. J. McNall and Halver C. VanDame, Cincinnati; Harry W. Conroy and Floyd E. Yarnall, Kansas City; Sidney Williams, Minneapolis; N. Aubrey Carson and Frederick M. Garfield, St. Louis. Acknowledgment is also due to William Weiss, Washington, D. C., for drafting the charts.

RECOMMENDATIONS*

It is recommended—

(1) That the statement in the cheese method for fat, 22.130, "Add ca 0.5 g of sand, previously digested with HCl, to prevent bumping . . ." be changed to read, "Add a few glass beads, or other inert material, previously digested with HCl, to prevent bumping, . . ."

(2) That study of methods for sampling, fat, and moisture in cheese be continued.

REPORT ON THE DETECTION OF ADDED WATER BY THE SERUM TESTS

By H. J. HOFFMANN (Department of Agriculture, Dairy & Food, St. Paul, Minnesota), *Associate Referee*

The Association of Food, Feed and Drug Officials of the South Central States, which met in April, 1946, adopted a resolution which reads as follows:

"The Food Committee recommends that a request be made to the Association of Official Agricultural Chemists for a study of the possibility of using the sour serum test for added water in milk as a quantitative procedure after re-investigating the qualitative limits now applying in that test."

The Committee submitted comments with this resolution which suggested the need of further information as to the value and use of the serum method in detecting added water in milk, in amounts of less than 13 per cent. They also feel that the sour serum limit of 38.3, which is now fixed in the official method, is too low for detecting or sorting out samples which contain added water in amounts of less than 13.5 per cent. They also state that some health departments are using the serum method in a quantitative manner. In response to the above resolution, and the added

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 52 (1949).

comments, the Associate Referee has completed certain work which it is hoped will serve to clarify this situation.

The tests outlined in the tables which are included in this report illustrate that the work has been performed on authentic samples of milk which were known to be free from added water. Other tests have been made where known amounts of water have been added to authentic samples, and the tests are complete with respect to serum results and detection of actual percentages of added water by the cryoscopic method.

DISCUSSION OF RESULTS

The writer was successful in obtaining only two collaborators to perform the necessary work which was requested for the 1948 meeting of the Association. The work, however, of these two collaborators has been excellent, and I think gives to this meeting the information which is desired. The Associate Referee feels that these results, coupled with the information which may be found in the literature, serve to demonstrate without question that the official serum methods only serve to indicate to the analyst that added water may be present in a milk sample. If all milk samples are reported as containing added water when they fall below the present standards (38.3 sour serum, 36 copper serum, and 39 acetic serum), the only thing the analyst is certain of is that the milk sample does contain added water. No indication is ever given as to any quantitative amount, and one is at a loss to understand the comments of the South Central Association of Food, Feed and Drug Officials, who state that "Some health departments are using the test in a quantitative manner." It would be very interesting to learn exactly who these health departments may be, so that through correspondence perhaps their results would be made available for study.

This department has maintained a great many successful prosecutions in the courts for the addition of added water in milk. In every instance it has been necessary to include as a part of the court records the exact percentage of added water. Courts and juries are loath to convict a law violator when it can not be established to their satisfaction exactly what the wrongdoer has done. In view of these facts, this office would not authorize prosecution based solely on a result obtained by any one of the serum methods, since we could never by testimony state to the court any known percentage of added water. We have had sufficient experience on this score to know that we can not secure a verdict in our favor, therefore do not prosecute unless cryoscopic results are available.

The presence of added water may be indicated to an analyst by several methods. Serum readings may be taken by any one of the three official methods, and if these readings are below the accepted standards of 39, in the case of acetic serum; 38.3 in the case of sour serum; or 36, in the case of copper serum, then the true percentage of water should be determined

TABLE 1.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER—NONE									
	FAT	S-N-F	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML		CRYSCOPIC FREEZING POINT—0°C.	EXAMINATION ADDED WATER
	per cent	per cent	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM		
A. Holstein Herd (15 cows)	3.10	8.79	42.5	42.0	38.4	None	.7812	.7488	.544	1.09
B. Predominately Brown Swiss, some Holstein (10 cows)	3.60	9.26	43.7	43.2	39.1	None	.7856	.7600	.542	1.45
C. Holstein Herd (8 cows)	2.85	8.46	41.4	41.9	38.1	None	.7780	.7465	.546	0.73
D. Mixed Herd (7 cows)	3.85	8.73	41.4	42.0	38.2	None	.7884	.7428	.557	None
E. Mixed Herd (12 cows)	3.35	9.07	42.7	42.7	38.2	None	.8060	.7636	.546	0.73

Note: In all the tables, the words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.

TABLE 2.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER, 5%									
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML					
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
A. Holstein Herd (15 cows)	41.1	40.6	37.4	None	.7416	.7108				
B. Predominately Brown Swiss, some Holstein (10 cows)	42.1	41.7	38.1	None	.7440	.7108				
C. Holstein Herd (8 cows)	39.7	40.5	37.1	None	.7360	.7088				
D. Mixed Herd (7 cows)	39.8	40.6	37.2	None	.7460	.7120				
E. Mixed Herd (12 cows)	40.9	41.3	37.2	None	.7604	.7296				

Note: The words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.

TABLE 3.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER, 10%					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
A. Holstein Herd (15 cows)	39.6	39.3	36.3	Probable	.7028	.6876
B. Predominately Brown Swiss, some Holstein (10 cows)	40.5	40.2	37.0	None	.7052	.6868
C. Holstein Herd (8 cows)	37.7	39.1	36.3	Probable	.6916	.6724
D. Mixed Herd (7 cows)	38.2	39.1	36.2	Probable	.7016	.6660
E. Mixed Herd (12 cows)	39.7	39.8	36.3	Probable	.7196	.7008

Note No. 1. The words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.

Note No. 2. Used the word "probable" for indicating added water when the reading of anyone of the three serums was suspicious.

TABLE 4.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER, 15%					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
A. Holstein Herd (15 cows)	37.6	38.1	35.3	Present	.6628	.6484
B. Predominately Brown Swiss, some Holstein (10 cows)	39.1	38.7	36.0	Probable	.6652	.6524
C. Holstein Herd (8 cows)	36.7	37.7	35.3	Present	.6568	.6332
D. Mixed Herd (7 cows)	36.9	37.8	35.2	Present	.6684	.6304
E. Mixed Herd (12 cows)	38.1	38.3	35.3	Present	.6856	.6580

Note No. 1. The words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.

Note No. 2. Used the word "probable" for indicating added water when the reading of anyone of the three serums was suspicious.

TABLE 5.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER, 20%					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
A. Holstein Herd (15 cows)	36.2	36.7	34.3	Present	.6232	.6108
B. Predominately Brown Swiss, some Holstein (10 cows)	37.5	37.2	35.0	Present	.6256	.6084
C. Holstein Herd (8 cows)	35.1	36.4	34.3	Present	.6128	.5904
D. Mixed Herd (7 cows)	35.4	36.4	34.2	Present	.6240	.5916
E. Mixed Herd (12 cows)	37.1	36.8	34.3	Present	.6336	.6128

Note No. 1. The words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.

TABLE 6.—*Investigation of the sour serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minnesota

DESCRIPTION AND NUMBER OF SAMPLE	MILK CONTAINING NO ADDED WATER					
	SP. GR. @60°F.	FAT per cent	S.N.F. per cent	SOUR SERUM* @20°C.	ADDED WATER INDICATED	SOUR SERUM ASH
Past. Milk No. 183	1.0311	3.5	8.61	40.00	None	.7662
Past. Milk No. 78	1.0321	3.5	8.87	40.00	None	.7712
Past. Milk No. 79	1.0317	3.7	8.80	39.32	None	.6440
Past. Milk No. 80	1.0322	3.6	8.91	40.72	None	.7720
Past. Milk No. 81	1.0318	3.6	8.82	40.35	None	.7648
Past. Milk No. 82	1.0321	3.5	8.87	41.00	None	.7632
Past. Milk No. 83	1.0321	3.6	8.89	41.33	None	.7756
Past. Milk No. 279	1.0321	3.3	8.81	41.52	None	.7708
Past. Milk No. 109	1.0322	2.9	8.77	41.03	None	.7684

* Immersion Refractometer Readings 20°C.

TABLE 7.—*Investigation of the sour serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minn.

DESCRIPTION AND NUMBER OF SAMPLE	WATERED MILK—SOUR SERUM READING 35.3 OR OVER					
	SP. GR. @60°F.	FAT per cent	S.N.F. per cent	SOUR SERUM* @20°C.	ADDED WATER INDICATED	SOUR SERUM ASH
Raw Milk No. 240	1.0302	2.7	8.22	38.71	None	.7042
Raw Milk No. 203	1.0313	3.0	8.56	39.48	None	.7362
Raw Milk No. 204	1.0319	3.0	8.70	40.00	None	.7346
Raw Milk No. 205	1.0317	3.5	8.76	39.75	None	.7314
Raw Milk No. 206	1.0311	3.0	8.50	39.31	None	.7310
Raw Milk No. 207	1.0311	2.8	8.48	39.82	None	.7302
Raw Milk No. 208	1.0321	2.7	8.70	39.79	None	.7328
Raw Milk No. 209	1.0312	3.25	8.57	39.18	None	.7310
Raw Milk No. 210	1.0314	3.6	8.71	39.46	None	.7344
Raw Milk No. 211	1.0294	3.9	8.28	39.80	None	.7320
Raw Milk No. 212	1.0313	3.25	8.62	40.00	None	.7420
Raw Milk No. 310	1.0285	3.3	7.91	38.41	None	.6964

* Immersion Refractometer Readings 20°C.

TABLE 10.—Investigation of the serum method for detection of added water in milk
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food,
St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER (5%)					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
Guernsey Herd	41.2	42.1	37.5	None	.7732	.7476
Market Milk Grade A	38.6	40.3	36.3	None	.6960	.7072
Market Milk Grade A	39.6	40.8	36.7	None	.7496	.7304

TABLE 11.—Investigation of the serum method for detection of added water in milk
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food,
St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER (10%)					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
Guernsey Herd	39.8	40.8	36.5	Probable	.7308	.7260
Market Milk Grade A	37.3	38.7	35.4	Present	.6840	.6720
Market Milk Grade A	38.0	39.3	35.7	Present	.6948	.6924

TABLE 12.—Investigation of the serum method for detection of added water in milk
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food,
St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER (15%)					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
Guernsey Herd	38.0	39.0	35.5	Present	.6860	.6856
Market Milk Grade A	36.0	37.2	34.3	Present	.6508	.6304
Market Milk Grade A	36.6	37.7	34.8	Present	.6708	.6480

TABLE 13.—Investigation of the serum method for detection of added water in milk
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food,
St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER (20%)					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
Guernsey Herd	36.6	37.6	34.4	Present	.6528	.6232
Market Milk Grade A	34.6	35.9	33.4	Present	.6064	.5932
Market Milk Grade A	35.2	36.3	33.7	Present	.6248	.6128

by the cryoscopic method, which will supply the analyst with the exact percentage of added water. The presence of added water may also be indicated to the analyst from the fat and solids-not-fat figure coupled with the graph according to Brown and Ekroth. It has also been found that the specific gravity figure, together with a comparison with the Brown and Ekroth table, is of value in a sorting procedure, and will indicate whether an official test be made by the cryoscopic method. It is very probable, however, that whether the analyst adopt any one of the above enumerated procedures (serum, specific gravity, or Brown and Ekroth table) that any one of these procedures only indicates whether added water may or may not be present. In no instance do any of these procedures give any quantitative results, which may only be obtained by the cryoscope.

From the figures in the tables which give the results for milk containing small (5-10%) percentages of added water, it will be apparent that present serum figures would fail to disclose the presence of such added water. Since the object of any one of the serum procedures is to detect the presence of added water in milk it must be apparent that the figures, 38.3 sour, 39 acetic, and 36 copper, must be at such a level as to give authentic and reliable information to the analyst. A further examination of all of the serum figures discloses that they are not uniform with respect to the presence of added water which has been detected by means of the cryoscope. This would serve to indicate that the serums do show which samples may contain added water, but do not give any true criterion as to the exact percentage present, inasmuch as the serums do not decrease in direct numerical order with respect to the true percentage of added water present in the milk sample. This discrepancy is true with respect to milks from like breeds of cows and becomes more apparent when the serums of milks from various breeds of cows are compared with one another. The sour serum is an analysis which is performed with extreme difficulty to-day. Most milk which is now offered to consumers is pasteurized, and when the analyst seeks to prepare a sour serum he frequently has the difficulty of having the milk rot, rather than sour. It has been the experience of analysts that sour serums are frequently only obtained when a culture is added to the milk, and this procedure is, of course, not provided for in the official method. This difficulty is not encountered when the copper and acetic serum tests are made.

I wish to take this opportunity to compliment Mr. Donald J. Mitchell, of the State Chemical Laboratory, at Vermillion, South Dakota, and Mr. H. C. Petersen, of the Associate Referee's staff. These two gentlemen, through their collaborative work, have brought to light the errors which probably exist in the present method, and have shown the need for new work which must be performed if these methods are to be used for giving a true picture as to the composition of milk samples.

RECOMMENDATIONS*

The Associate Referee recommends that the serum procedures be confined to their present limitations, *i.e.*, that they serve to be used as a yardstick to indicate the presence of suspected samples. In view of the results obtained, however, it would seem that the present serum figures of 38.3 for the sour serum, 39 for the acetic serum, and 36 for the copper serum are subject to question. It is therefore recommended:

(1) That further study be made with the view in mind of raising these present figures to some higher level, with the object in mind of causing the method to be more effective in screening out suspected watered milk samples. In the light of present information, I do not like to recommend any definite figure, but suggest that a level of 40 for the sour serum, 40 for the acetic serum and 37 for the copper serum be used as a basis for the further study which I recommend in this report.

(2) That efforts be abandoned to make any one of the serum methods applicable to any quantitative procedure, since the results indicate that this goal can not be achieved.

(3) That the present official copper (22.30) and acetic serum (22.28) methods be made tentative until sufficient new data is available and that the present sour serum (22.29) be dropped completely, since it has the same analytical defect as do the other serum methods and is a determination which is extremely difficult to perform due to the change in methods of marketing milk.

No reports were given on phosphatase test in dairy products, ash in milk and evaporated milk, frozen desserts, chlorine in milk, preparation of butter samples, tests for reconstituted milk, acidity of milk, or fat in dairy products.

REPORT ON MICROANALYTICAL METHODS FOR
EXTRANEOUS MATERIALS IN FOODS AND DRUGS

By KENTON L. HARRIS (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The methods at present in Chapter 42 of the sixth edition of *Methods of Analysis* resulted from an attempt to cover a wide range of products in a relatively new field. In line with this same thought the Association adopted several additional tentative procedures at the 1945, 1946, and 1947 meetings. We now find ourselves in a position where, along with the addition of methods that normally may be expected, we can replace several specific procedures by generalized directions that cover a whole group of products. Such is the case with 42.54 (Candy). In place of eight procedures

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 53 (1949).

we now recommend that three more universally applicable methods be applied. So far as Chapter 42 is concerned, a consolidation of methods will be an aid to the interpretation of comparative results from different products. It is planned to continue this trend following out the recommendations of the Committee on Classification of Methods (*This Journal*, Feb. 1948) and work toward the inclusion of generally applicable official methods.

The Associate Referee assignments should be regrouped as follows for 1949:

Extraneous Materials in Baked Products, Cereals, and Confectionery.

Extraneous Materials in Nut Products.

Extraneous Materials in Fruit Products.

Extraneous Materials in Dairy and Egg Products.

Extraneous Materials in Beverage Materials.

Extraneous Material in Vegetable Products.

Extraneous Materials in Drugs, Spices, and Miscellaneous Products.

Sediment Tests for Milk and Cream should be included in this section for Extraneous Materials.

RECOMMENDATIONS*

The new methods for pepper and prepared mustard have been developed to the point where collaborative work is needed. It is recommended that work be done on them this coming year.

The reports of the Associate Referee for Nut Products and Confectionery and the Associate Referee for Baked Products, Cereals, and Eggs contain completely new methods for candy and for baked products, which have been used successfully by analysts of some U. S. Food & Drug Administration stations. It is recommended that collaborative results be obtained on them as soon as possible.

The procedure suggested to replace the present method for starch, 42.38, clarifies several aspects of the old method and is recommended for tentative adoption.

The recommendations on sections 42.29–42.31 are based upon the work by K. L. Harris, *This Journal*, 31, 786 (1948) and the observations of many analysts which indicated a need for an alternative HCl-boil flour method and the necessity for several changes in the pancreatin procedure. It is recommended that the collaborative work on these methods be continued.

The two new procedures suggested for popcorn fill a vacancy that has long existed. It is recommended that these methods be accepted as tentative procedures.

Method 42.32 for rodent excreta, in corn meal, is recommended for official, final action.

The methods for mold in cranberry sauces, *This Journal*, 31, 783 (1948), are recommended for official, first action; and the suggested strawberry

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 53 (1949).

procedures of the Associate Referee for Fruit Products and Beverage Materials, are recommended for further study.

It is recommended that the changes in the wording of 42.61(b) and 42.75 be made.¹

REPORT ON EXTRANEOUS MATERIALS IN DRUGS, SPICES, AND MISCELLANEOUS MATERIALS

By WILLIAM V. EISENBERG (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Because of the undue amount of time involved in picking over pepper berries, two semi-mechanical sorting procedures were studied. The method for "siftings and pickings" was submitted by Eastern District

TABLE 1.—*Comparison of whole black pepper separation by flotation in alcohol and kerosene-petroleum ether*

SAMPLE	PERCENTAGE WEIGHT OF FLOATERS IN—	
	95% ALCOHOL	KEROSENE-PET. ETHER (sp. gr. 0.800)
1	2.40	3.80
2	3.20	2.40
3	3.14	1.86
4	1.50	1.60
5	4.96	6.40
6	7.70	7.60
7	3.30	3.60
8	6.00	4.80
Average	4.03	4.01

of the U. S. Food and Drug Administration, where the method has been in use for many years. The procedure employs a standard pepper sieve known as a No. 9½ round-hole sieve. Because this sieve is not manufactured in this country and is obtainable only in England, a detailed description is appended to the method.

The gravity flotation procedure was submitted by Powers and Mendelsohn from the San Francisco and Los Angeles Stations, respectively, of the U. S. Food and Drug Administration. The method is based on the fact that peppers which are internally moldy, decomposed, or insect infested are lighter than sound peppers and may be separated by flotation in liquids of suitable specific gravity, *i.e.*, about 0.80. Both 95% alcohol and 0.800 sp. gr. kerosene (the latter adjusted to this specific gravity, with petroleum ether or gasoline if necessary) were proposed as suitable

¹ For details of changes, see *This Journal*, 32, 117 (1949).

liquids. Data by Eisenberg and Ungar comparing the separation by the two liquids indicates that there is very little significant difference in the use of either liquid (Table 1).

The gravity flotation method is intended to supplant in part the "dry-cut" procedure which entails cutting open each pepper berry for visual examination. Comparative data by Mendelsohn indicates that the separation by flotation with kerosene-petroleum ether shows no significant difference from the dry-cut procedure (Table 2). The data show that

TABLE 2.—*Comparison of whole black pepper separation by flotation and by dry-cut procedures*

SAMPLE*	WEIGHT DAMAGED—PER CENT		NUMBER DAMAGED—PER CENT	
	DRY-CUT	KEROSENE-PET. ETHER	DRY-CUT	KEROSENE-PET. ETHER
1	4.00	2.80	8.53	7.05
2	4.40	4.84	9.76	8.77
3	2.90	3.50	7.26	8.29
4	3.20	2.40	3.05	3.51
	—	—	—	—
	3.63	3.39	7.15	6.91

* 25 g or about 750 berries in each test.

the two methods are in closer agreement when compared by count than by weight. This is readily understandable because of the lightness of the "floaters" and the inordinate effect of an occasional damaged berry that may be heavy enough to sink in the liquid. The flotation technic is less time-consuming and should therefore make feasible the examination of a larger and more representative sample. The peppers that float are subsequently cut open for examination to determine the kind and extent of damage. Samples involving external damage which has not penetrated the berry, such as surface mold, will not be suitable for examination by the new procedure, and the dry-cut method will be retained for such samples.

The methods for whole black pepper follow:

WHOLE BLACK PEPPER

(A) *Siftings and Pickings*

Standard pepper sieve.—Use the so-called "No. 9½ round hole sieve" specified in the Rules for the Standard Arrival Contract of the American Spice Trade Association. It consists of a round screen with frame 18–22" in diameter and 2½" in height (small or "office" size 8 or 9" in diameter); bottom made of metal, perforated with round holes averaging 0.112" in diameter (approximately 7/64"); average 5½ holes to the linear inch.

(a) *Siftings.*—Weigh sample to the nearest ounce and sift entire sample, consisting of not less than 5 lbs. Introduce 1½ to 2 lbs. of pepper into the sieve at a time; (when using the small or "office" size sieve, introduce only ¾ to 1 lb. into the sieve at a time). Obtain the siftings by tilting the sieve from side to side, so that the pepper

passes from one side of the screen to the opposite side 10 times. Weigh siftings and report as percentage siftings.

(b) *Pickings*.—Examine sifted sample for foreign material, consisting of stems, stones, mud, and other foreign matter. Add the pickings to the siftings, weigh the combined total, and report as percentage siftings and pickings.

(B) *Separation of Damaged Berries by Flotation*

Weigh 50 g of pepper, count, and transfer to a tall 800 ml beaker about $\frac{3}{4}$ filled with 95% alcohol or kerosene-petroleum ether mixture (sp. gr. 0.800). Stir ca 1 min. with spoon. Skim off the floating berries, dry, count, and weigh. Record per cent of floating berries by count and by weight. Cut open representative number of floating berries, determine and report kind and extent of damage.

Method 42.94, which has been found to be unsuitable for most prepared mustards, should be replaced by a new method submitted by the Cincinnati Station of the U. S. Food and Drug Administration. This new method, which proposes a HCl digestion in place of the pancreatin digestion, serves to simplify the procedure and results in a better filth separation.

The method follows:

PREPARED MUSTARD

Filth

Transfer 100 g to a 2-liter trap flask. Add 500 ml H₂O and 15 ml concd HCl. Boil for 10–15 min., stirring constantly to prevent scorching. Cool. Trap off using gasoline and H₂O.

No report was given on extraneous materials in dairy products.

REPORT ON EXTRANEEOUS MATERIALS IN NUT PRODUCTS AND CONFECTIONERY

By W. G. HELSEL (Food and Drug Administration, Federal Security Agency, Washington, C. D.), *Associate Referee*

The present (42.54) methods for filth in candy have not been universally applicable to the wide variety of candy products now on the market and it is recommended* that the following procedures which were developed by the analysts of the Baltimore and San Francisco Stations of the Food and Drug Administration be studied.

"Filtration of hard candy, gum drops, gum, starch, or pectin-base candies. Dissolve in boiling 0.5% HCl (0.5+35) and filter thru rapid filter paper in suction funnel. Examine filter for filth." 42.54 (b). Substitute the following for 42.54 (b), (c), (d), (e), (f) and the paragraphs beginning "In candy containing corn flakes . . ." and "In chocolate candy coating . . .":

"Chocolate candy with or without fruit or nuts, fruit candy, etc.—Weigh 225 g of sample into a 2-liter beaker. Add 1 liter 5–10% Na₂B₄O₇ soln. Simmer 10–15 min.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

Pour thru 5-8" #140 screen. While pouring play a forcible stream of hot water on this material. Wash well with large stream of hot water. Wash twice alternately with alcohol and CHCl_3 in that order and give a final alcohol rinse. Transfer material to a filter paper if little residue remains or to a 1-2 liter Wildman trap flask if a larger amount remains. Transfer bulk of material with a spoon. Rinse residue from screen with alcohol from a wash bottle. Wash screen with a forcible stream of hot water, collecting final residue at one edge of screen and transferring to the trap flask with a stream of alcohol as above. Add 200 or 400 ml of 60% alcohol depending on size of trap flask. Boil for 20 min. Cool below 20° and add 20 or 40 ml gasoline. Fill flask with 60% alcohol and trap off in usual manner. Add gasoline and trap off a second time. Filter and examine. If a large amount of peanut testa or similar material floats up into the neck of flask, pour trapped-off material thru a #8 or #10 sieve, rinse thoroly, filter liquid portion, and examine."

(c) "*Chocolate candy coating for insect excreta and other filth.*—Heat 400 ml gasoline in an 800 ml beaker to $40-50^\circ$ and maintain at this temp. Place a portion of candy in a wire basket (ca $3\frac{1}{4}$ " diam. \times 1" high) made from #8 screen and with wire handles. Move basket up and down thru the gasoline until chocolate coating is dissolved. Rinse each candy center with a fine stream of gasoline from a wash bottle and save the center. Repeat with balance of sample. Stir the gasoline-chocolate suspension and pour thru a #140 sieve. Transfer residue from the sieve to a filter paper and examine microscopically. Examine the candy centers by 42.54 (a) or (b) as given above

REPORT ON EXTRANEEOUS MATERIALS IN BAKED PRODUCTS, CEREALS, AND EGG PRODUCTS

By J. FRANK NICHOLSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In view of the widespread difficulties encountered in using the 42.26-42.28 methods on baked products it is believed that these present methods should not be used and that the following procedures, which have been used successfully for several years, should be substituted for them and studied with a view to official adoption.

(1) *Sieving for insect fragments and rodent hairs.*—Weigh 225 g of sample into a 2 liter beaker. Add sufficient hot water to soften and saturate the material. If lumps persist or if water is not immediately absorbed uniformly thru the entire mass (e.g., in the case of hard English-type cookies and alimentary paste) estimate volume of mixture and adjust the acidity to 1% by adding sufficient concd. HCl (1 part concd. HCl to 35 parts of mixture). Boil until mixture becomes finely divided and so digested that it will not froth over when covered during boiling. Neutralize to ca pH 6 with NaOH soln. Add Na_2PO_4 soln to bring mixture to pH 7-8. Stir and break up the material as much as possible. Cool to 40° , add 100 ml of pancreatin soln made as directed in 42.2(c) and then diluted to 100 ml. Stir thoroly and readjust to pH 7-8. Allow to stand 30 min. Stir and readjust pH.

For white flour products, add 2 ml of formaldehyde and digest overnight. For products made from whole wheat and rye flours and similar materials of high bran content, digest only 2-3 hours. (The shorter digestion is advisable because the starch and protein are not completely stripped from the bran and gives a cleaner separation on trapping.)

After digestion pour this material thru 5-8" #140 screen. While pouring play a

forcible stream of hot water from the tap on this material. Wash well with a large stream of hot water. After complete washing (no starchy material visible unattached to bran) wash twice alternately with alcohol and chloroform in that order, and give a final alcohol rinse.

Transfer the material to a filter paper if little residue remains or to a 1-2 liter Wildman trap flask if a larger amount remains. Transfer the bulk of the material with spoon. Rinse residue from the screen with alcohol from a wash bottle. Wash screen with a forcible stream of hot water, collecting final residue at one edge of screen and transferring to trap flask with a stream of alcohol as above. Add 200 or 400 ml of 60% alcohol depending on the size of the trap flask. Boil for 20 min. Cool below 20° and add 20 or 40 ml of gasoline, fill flask with 60% alcohol and trap off in usual manner. Trap off a second time. Use care in stirring and during addition of alcohol to prevent formation of emulsion or inclusion of air. If residue in the flask tends to rise, stir material down 2-3 times. Filter trapped-off material and examine.

(2) *Direct trapping to show insect fragments and rodent hairs contributed by wheat or mill.*—To 1000 ml of boiling 1% HCl add 225 g sample. Continue heating for 30-40 min., or until the mixture becomes a finely divided mass that will not froth over when covered; cool somewhat. Neutralize with NaOH soln and bring to pH 7-8 with Na_2PO_4 soln. Cool to 35-40° and digest with pancreatin as in (1) above. Bring to a boil; cool; transfer to 2-liter Wildman trap flask, and extract and examine in the usual manner.

The above method was first used by Baltimore Station (U. S. Food and Drug Administration) and later modified by use and by other stations. This method is an attempt to standardize the bakery products methods so as to insure more uniform results.

RECOMMENDATIONS*

It is recommended—

(1) That method 42.38 for starch, which contains several ambiguities, be deleted and a method, which is essentially a clarified revision of the same procedure, substituted as a tentative method.¹

Weigh out 225 g of starch into a 1500 ml beaker. Add with stirring 1200 ml of cold water (15-20°). Stir out lumps and pour thru 5-8" No. 140 sieve. Wash with cold running water. Rinse particles from the sieve to a filter paper, using first water and then 60% alcohol. Examine paper microscopically.

This change was suggested by Mr. E. M. Hoshall of Baltimore Station, U. S. Food and Drug Administration, to clarify certain portions of the procedure and to standardize it with other methods of this same type.

(2) That sentence in parentheses, in method 42.31(b) for flour, be deleted; and that 42.29 and 42.30 be deleted and the following methods substituted:

Insect fragments and rodent hairs (Two alternates):

(a) *Pancreatin digestion.*—Weigh 50 g of flour into beaker and stir into a thin smooth slurry. Add a filtered aqueous extract of 5 g pancreatin and mix. Adjust to

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

¹ Details of the methods are published in *This Journal*, 32, 116 (1949).

pH 7-8 with Na_3PO_4 soln. Allow to stand 15 min., and if necessary readjust to pH 7-8. Digest overnight. Transfer digested material to 2 liter Wildman trap flask. Trap off twice with gasoline and water in the usual manner. Combine trappings in beaker. Transfer contents of beaker to trap flask and fill with H_2O . Stir and after 30 min. trap off into beaker and filter thru rapid filter paper using suction. Examine microscopically.

(b) *HCl digestion*.—To 50 g flour add water and stir into a thin smooth paste. Add water to make total 400 ml. Add 17 ml concd. HCl. With intermittent stirring bring to a boil and boil 10 min. Cool (if in a beaker transfer to 2-liter trap flask), trap off with gasoline and water in the usual manner. Combine trappings in beaker, filter, and examine.

These changes are based on work to be reported in *This Journal* by Kenton L. Harris, Division of Microbiology, U. S. Food and Drug Administration.

(3) That two new methods on filth in popcorn be adopted as tentative methods.¹

The method for unpopped popcorn was developed by analysts of Chicago and Minneapolis Stations, U. S. Food and Drug Administration. The method for popped popcorn was developed by Mr. R. T. Elliott of Seattle District, U. S. Food and Drug Administration.

(4) That method 42.32, which was made official, first action, last year, be adopted as official, final action.

REPORT ON SEDIMENT TESTS IN MILK AND CREAM

By CURTIS R. JOINER (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

In 1929 Hoffmann (1) presented a method for the preparation of standard sediment pads using weathered cow dung suspended in 50 per cent sugar solution. On the basis of a series of pads prepared by each of seven collaborators, he recommended that the method be adopted as tentative. However, Subcommittee C recommended (2) "That the method presented by the associate referee . . . be further studied, and that the associate referee confer with other associations interested in this line of work whose methods are now uniform with those of this association, with a view of preserving the existing uniformity." This recommendation was repeated by Subcommittee C in 1930 and in 1931.

In 1935 Frary (3) recommended "that this association adopt the method for sediment test described in *Standard Methods of Milk Analysis*, 1934, 6th ed., pages 44-46, as tentative." Subcommittee C concurred in this recommendation. The method as adopted was essentially that proposed by Hoffmann.

J. O. Clarke, then Chief of the Central District, Food and Drug Administration, in 1943 was a member of a committee appointed by the

American Public Health Association to standardize methods of sediment testing. At his request, Samuel Alfend of the St. Louis Station prepared several series of standard sediment pads* using peat moss, dried cow manure, and a mixture of cow manure, soil, and charcoal. Several persons experienced in making milk sediment tests agreed that the pads made from the mixture of materials more closely resembled pads obtained from commercial milk than did those prepared from peat moss and cow manure alone. It was recognized, however, that the experimental pads did not resemble many commercial milk sediment pads at all.

The committee examined the discs prepared by Alfend and some prepared by another member of the committee. In addition, Clarke had six different laboratories make up a series of pads by Alfend's method (except that they were directed to filter the sediment through pads with the aid of suction, whereas Alfend drew the sediment and milk up into a milk sediment pump and forced the milk out through the pad), using materials from local sources, and submit them to the committee for study. The committee finally recommended that the method developed by Alfend (modified to filter the milk by suction) be adopted by the A.P.H.A.

In 1947 Frary (4) recommended that the tentative method for sediment test in milk be dropped, and that the A.P.H.A. method (5) be adopted as tentative. Subcommittee C concurred and recommended that the method be further studied.

In using the method as written, some workers have had difficulty in getting the sediment to remain in uniform suspension long enough to withdraw a representative aliquot with a pipet. The soil and manure particles have a tendency to settle out, while the larger pieces of charcoal rise to the surface. Prior to his appointment as Associate Referee, the writer used a motor stirrer at low speed to stir the sediment suspension while withdrawing aliquots. This stirring appeared to overcome the above difficulties. For measuring aliquots, it is convenient to use a series of four graduated pipets (5, 10, 25, and 50 ml.) with the tips cut off so the openings have a diameter of two to three millimeters. The method as used by the writer is given below, the italicized portions being changes or additions to the present tentative method:

(d) *Preparation of standard sediment discs.*—Make a uniform mixture of oven dried (100°) materials which meet the following screening specifications:

	<i>Per cent</i>
Cow manure, 40 mesh.	53
Cow manure, 20 mesh, retained on 40 mesh.	2
Garden soil, 40 mesh.	27
Charcoal, 40 mesh.	14
Charcoal, 20 mesh, retained on 40 mesh.	4

* Unpublished work.

Place 2.00 gm. of the above mixture in a 100 ml volumetric flask and moisten with 5 ml of 1% aerosol soln or other suitable wetting agent, add 46 ml. of 0.75% gum soln such as gum arabic (42.2(b) (1)), and then make up to 100 ml with a 50% by weight sucrose soln. Mix thoroly, pour into a 250 ml beaker or container of similar size and stir with a small motor stirrer at a speed (ca 200-300 r.p.m.) such that the mixture is thoroly agitated, but so that very little air is whipped into the suspension.

Transfer (while stirring) a 10 ml portion (200 mg of standard sediment) with a large tipped, graduated pipet to a flask and make up to a liter with 50% by weight sucrose soln. When thoroly mixed, each ml contains 0.2 mg of sediment. Mix contents of flask, pour into 1500 ml beaker and stir with suitable motor stirrer as directed above. While stirring, pipet out definite volumes of the sediment mixture and add to $\frac{1}{2}$ pint of filtered skimmed milk. Mix thoroly and pass the mixture thru a standard sediment disc in filtering device having a filtering area measuring $1\frac{1}{2}$ in. in diam. Pour the milk gently down the side of filtering apparatus and filter with very little or no suction. Wash the container promptly with $\frac{1}{2}$ pint of filtered skimmed milk. Let the last portion of the milk flow thru the pad with no suction applied. If sediment does not appear to be evenly distributed over pad, add 15 or 20 ml of milk and let it filter thru without any suction. Repeat this until sediment appears to be evenly distributed. Suck air thru disc for ca 1 min. to remove excess milk. For a permanent record, mount and spray the disc with 40% formaldehyde or with a soln containing 2.5 g menthol and 2.5 g thymol made up to 100 ml with alcohol. Alternatively, if most of milk is removed by thoro aspiration, no preservative need be added to pads. A transparent colorless plastic cement may be used for mounting them. Following the above method, prepare a series of discs containing the sediment remaining from 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, and 14.0 mg. of the standard mixture. Identify each disc on the permanent record with the amount of sediment in milligrams used to prepare the pad.

(For the purpose of comparison, the entire series of discs may be used. . . .)

EXPERIMENTAL

The experimental work this year was limited to the following objectives:

1. To demonstrate the precision obtainable by one worker with a given sediment mixture.
2. To determine the difference in appearance between two sets of standard discs prepared from materials obtained from different geographical locations.

Two different sediment mixtures, designated A and B, were prepared from materials obtained as follows:

A. Fresh cow manure and soil from a dairy farm in Southeastern Louisiana. The manure was dried in the laboratory a few days before it was used.

B. Fresh cow manure obtained from a dairy farm in the St. Louis area in 1942 and dried in the laboratory. Soil from downtown St. Louis. The same lot of commercial charcoal was used in both A and B.

Twenty-one pads, made up as follows, were prepared from both A and B: 0.5, 3.0, 6.0, and 12.0 mg pads in triplicate, and single 1.0, 2.0, 4.0, 5.0, 7.0, 8.0, 9.0, 10.0, and 14.0 mg pads. The discs were cemented on two 8 by 10 inch cards and photographs were made. The two photographs are submitted as Figures 1 and 2.

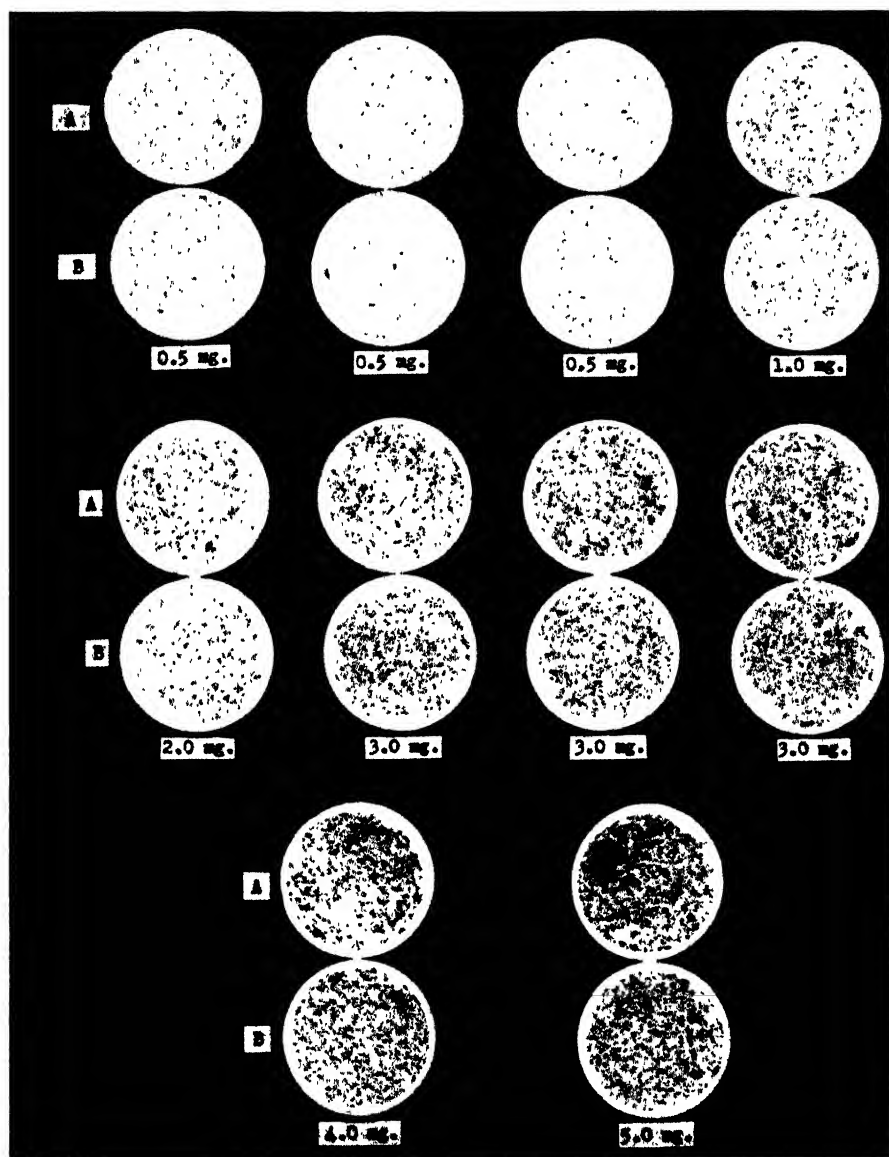


FIGURE 1.

Both the soil and the manure used in B appeared darker than those used in A. This difference in appearance was also apparent on the standard pads. To the eye in daylight, the predominating color of the B discs was gray-black, with some brown, while the A pads appeared to be predominantly brown with some black particles. The particle density on the two sets of discs appeared to be about equal, but the average particle size of A

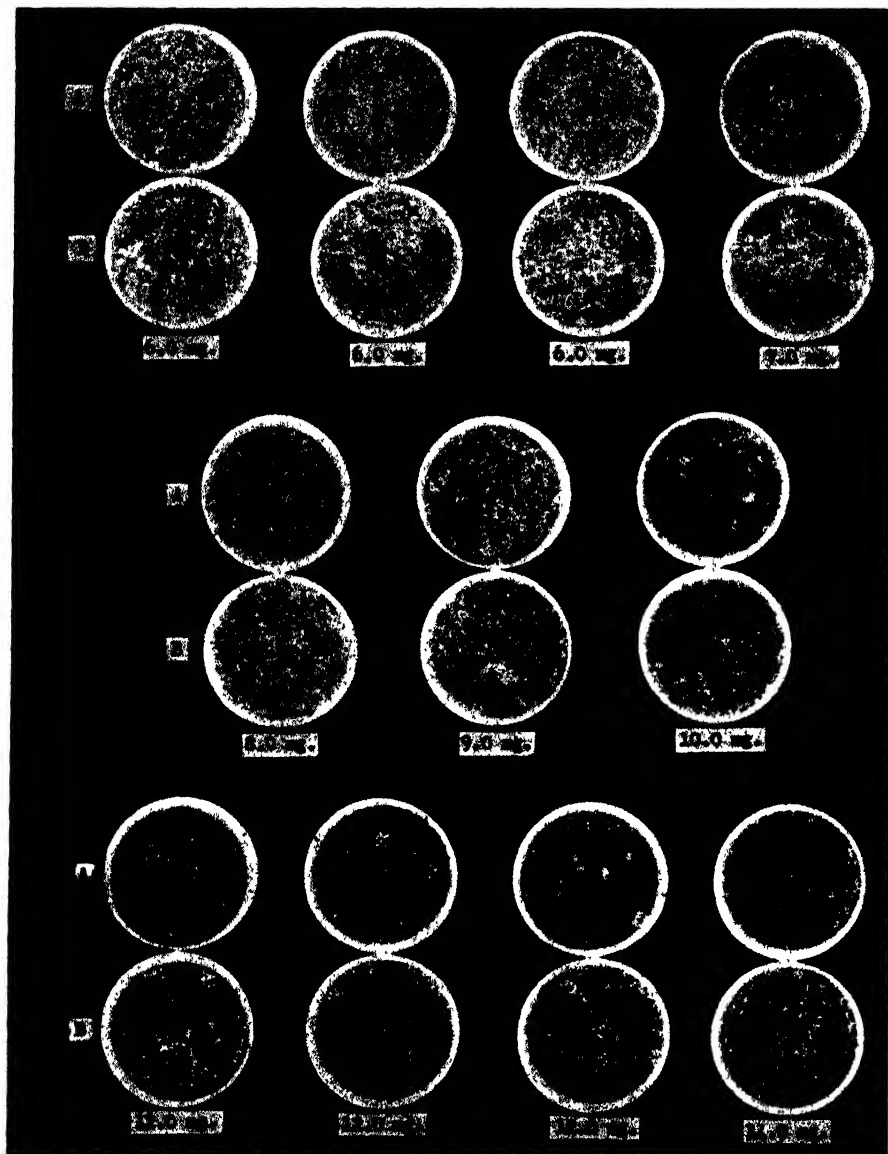


FIGURE 2.

seemed to be somewhat greater than that of B. The differences between the two sets of pads were emphasized in the photographs, and, in addition, the brown sediment on A photographed darker than did the gray-black sediment on the B discs. This difference is not noticeable with the 0.5 mg discs but with the 12.0 and 14.0 mg pads it becomes quite marked. In the photograph, the amount of sediment on the 14.0 mg B disc appears to be

slightly less than that on the 12.0 mg A pad. When viewing the actual pads, the former appears to fall in between the 12.0 and 14.0 mg A standard discs.

The various triplicate sets of pads are, for practical purposes, identical. In both groups of discs, in going from 0.5 mg to 14.0 mg there is, with one exception, approximately the proper proportional increase in the amount of sediment on the pads. The single exception is in the A set. The amount of sediment on the 8.0 mg disc appears to be equal to or slightly greater than the amount on the 9.0 mg pad. (In making these pads, no discs were discarded. In preparing a standard set of pads, any that are obviously out of line should be discarded and remade.)

In addition to the work represented by Figures 1 and 2, the Associate Referee made two diluted suspensions of sediment from one concentrated suspension and prepared a series of pads from each dilution. The two sets of pads were comparable in appearance.

DISCUSSION

The changes in the tentative method for the preparation of standard sediment discs presented in this report appear to have obviated the source of difficulties reported by some workers.* It is probable that standard pads prepared from sediment materials obtained from several different sections of the country would show greater variations than do the two sets of pads represented by Figures 1 and 2. If the variations in appearance of soil and manure from different sections of the country, or even within one area, are great enough to warrant it, it might be desirable for this Association to prepare a large batch of a standard sediment mixture for distribution.

The present tentative method for sediment in fluid milk (5) permits the use of photographic standards as a guide in grading sediment pads but recommends the use of actual standard discs. It is generally agreed by inspectors experienced in making milk sediment tests that pads are difficult to grade with photographic standards. However, it is very doubtful that actual standard pads could be used in the field unless the pads were rendered "permanent" by treating them in some manner that would prevent the sediment particles from falling off the pads. The Associate Referee has seen such a set of standard discs. They apparently had been treated with a solution of a transparent plastic in a volatile solvent. The Associate Referee has done a limited amount of work in the treatment of standard pads in this manner. The appearance of the pads are altered but slightly by this treatment. They are much more realistic appearing than are photographic reproductions.

SUMMARY

1. In the hands of the Associate Referee the method as presented in this report gives reproducible results with a given sediment mixture.

* Unpublished work.

2. There is considerable difference in the appearance of pads prepared from sediment mixtures from two different sections of the country.

3. The differences between standard pads are in some cases accentuated in photographic reproductions.

RECOMMENDATIONS*

It is recommended that—

(1) The method presented in this report be subjected to collaborative study in order to determine (a) the precision of the method in the hands of different analysts using the same sediment mixture, and (b) the variation in appearance of pads prepared with sediment materials from different sections of the country.

(2) Further work be done on the preparation and use of standard sediment pads rendered "permanent" by treatment with a solution of a transparent plastic.

REFERENCES

- (1) HOFFMANN, H., JR., *This Journal*, 13, 237 (1930).
- (2) Report of Subcommittee C on Recommendations of Referees, *Ibid.*, 67.
- (3) FRARY, GUY G., *Ibid.*, 19, 377 (1936).
- (4) ———, *Ibid.*, 31, 298 (1948).
- (5) Report on Changes in Methods of Analysis, *Ibid.*, 93.

REPORT ON EXTRANEEOUS MATERIALS IN FRUIT PRODUCTS AND BEVERAGE MATERIALS

By F. ALLEN HODGES (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

There are included below two new procedures for cranberry sauce which cover a field where previously there has been no established method. This method, *This Journal*, 31, 783 (1948), has been subjected to collaborative study and it is recommended for adoption as official.

Several difficulties have been reported in the 42.41 and 42.42 procedures for frozen strawberries. As a result of investigations by W. G. Helsel, U. S. Food and Drug Administration, it is now possible to recommend changes in the strawberry procedures. The draining of sliced strawberries is unnecessary under present commercial practices in which practically all berries are packed in either 30-pound cans or consumer-sized packages which freeze more uniformly than the larger barrels. Since the sugar is no longer removed, the suction-alcohol treatment is needed to remove air. The larger sieve opening permits the increase in sample size and yet will retain rotten berry tissue well under 6 mm in diameter. The new procedure is given below and should be further studied.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

42.41

PREPARATION OF SAMPLE

Each sample representing a barrel, 30 lb. can, or other large container, should consist of ca 4 quarts of berries. If the berries are packed in 1 lb. consumer packages, four such packages from the same code should be composited to make one sample. Thaw the entire sample and divide into 2 portions including juice, without damaging berries.

42.42

ROT

(a) *Mold count*.—Pulp one portion of sample thru cyclone with screen openings 0.027" in diam. and mix thoroly (pour juice thru cyclone first). If the pulp contains too many air bubbles for mold counting, mix thoroly ca 50 g of pulp and 1 ml alcohol. Remove most of the air from mixture with suction, mix thoroly, and make mold count as directed under 42.57.

(b) *Macroscopic separation*.—Drain second portion of sample on No. 8 sieve. Immerse berries in tap H₂O in large white pan. Decant most of H₂O thru sieve, catch and return any strawberry tissue. Repeat washing until H₂O is fairly clear. Examine berries under H₂O and remove all questionable berries to another pan containing deaerated H₂O. Re-examine suspected berries. Confirm all questionable rot spots by examining fragments of berry tissue for mold under compound microscope. Classify tissue as rotten only when a substantial number of mold filaments are present. Class a berry or fragment as rotten if it has rot area 6 mm. or more in diam. Separate berries and fragments with rot areas over 12 mm in diam. Drain separately the good and rotten berries 2 min. on No. 8 sieve and weigh.

REPORT ON EXTRANEIOUS MATERIALS IN VEGETABLE PRODUCTS

By FRANK R. SMITH (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The following changes are recommended* in the methods for extraneous materials in vegetable products.

42.61 (b).—Change separator size to 6 liter. The 5 liter size is not available in stock.

42.75 To clarify an ambiguity, add to the next to the last sentence ending "mushrooms" the words "allowing them to drop through the screen."

REPORT ON FISH AND OTHER MARINE PRODUCTS

By ANDREW M. ALLISON (Food and Drug Administration, Federal Security Agency, Boston 10, Mass.), *Referee*

In accordance with recommendations of the Committee on Classification of Methods (*This Journal*, 31, 63) the Referee has reviewed Chapter 24, Fish and Other Marine Products, in *Methods of Analysis*. As a result the following comments and recommendations for study are made. Discussion of current collaborative problems now under study and appropriate recommendations are also included in this report.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

The established shucked oyster standards and the proposed standards for shucked soft shell clams make it imperative that the applicable analytical methods in the Book of Methods be in conformity with those needed for the official standards. In the following comment we will mention specific points in connection with the revision and extension of such methods.

The paragraph (24.1, p. 359) "Apparatus-Tentative" does not completely meet the needs of present laboratory technics. For example: the skimmer described is smaller than that required for the measurements specified in making official oyster standard, drained liquid measurements. There are other descriptions in this paragraph warranting study and revision. It may be considered desirable to delete this paragraph and incorporate pertinent portions in other sections under appropriate methods.

Paragraph 24.2 on "Preliminary Treatment and Preparation of Sample-Tentative" needs to be thoroughly considered as to title and subject matter for possible revision of the wording in several places. For example: the part designated as (f) shucked oysters, indirectly suggests it is a method for the determination of free liquid by loss of volume, whereas the official standards require measurements in terms of loss of weight. Consideration should be given to eliminating the ambiguity of including what appears to be a method where it does not conform to the title of the paragraph. The method for determination of free liquid for shucked oysters and clams should be considered for preparation of a new separate paragraph in which could be included most of the description (which should be revised) now in paragraph 24.1. The method for drained liquid as outlined in the present oyster standard may warrant inclusion, as is, with appropriate changes in wording to fit the style of *The Journal* and the *Methods of Analysis*.

Although designated by Subcommittee C for study during the past year (*This Journal*, 31, 52) no work has been possible on Total Solids in Fish and Other Seafoods by the Associate Referee because of the press of other work.

Although no adverse reports have been received by the Referee on the method for Salt II (paragraph 24.7) "With Calcium Acetate as Fixative—Tentative," it is believed that the method should be studied for deletion if not needed, otherwise it should be considered with or without further collaborative study for official, first action. The method, if retained, could be condensed by omitting the last part and referring to the identical portion of paragraph 24.6.

The Associate Referee on Ether Extract in Fish has during the past year followed the suggestions of Committee C (*This Journal*, 31, 52) and has made studies of the composition of substances extracted from fish by ether, and by ether-petroleum ether mixture after acid hydrolysis. The report by Associate Referee Voth is being submitted.

RECOMMENDATIONS*

It is recommended—

(1) That paragraph 24.1 titled "Apparatus—Tentative" of *Methods of Analysis* be reviewed and studied for the purpose of revision.

(2) That paragraph 24.2 titled "Preliminary Treatment and Preparation of Sample—Tentative" of the *Methods of Analysis* be likewise reviewed and studied for the purpose of revision.

(3) That in connection with Recommendations No. 1 and No. 2 a study be made of methods for determining drained liquid in oysters and clams.

(4) That the subject, "Total Solids in Fish and Other Seafoods" be continued as a subject of study with the end in view of revising paragraph 24.3, titled "Total Solids—Tentative," which is now limited to oysters and scallops.

(5) That Method II for Salt (Chlorine as Sodium Chloride) paragraph 24.7, titled "With Calcium Acetate as Fixative—Tentative" in the *Methods of Analysis*, be studied for deletion (if no longer of value) or for revising and consideration of changing the "tentative" designation to "official, first action."

(6) That the extract obtained by using a mixture of ethyl ether and petroleum ether by the method described in *This Journal*, 31, 334 (1948), be designated as "Crude Fat (acid hydrolysis method)" and that the method so designated be adopted as official, first action.

REPORT ON ETHER EXTRACT IN FISH

By MENNO D. VOTH (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

The recommendation of Subcommittee "C" (*This Journal*, 31, 52, 1948) was that some further studies be made on ether extract in fish with the idea of determining whether the material, extracted by the solvents ethyl ether or a mixture of ethyl ether and petroleum ether, is largely fat.

Two separate quantities of extract were prepared. In both cases the fat was extracted from canned mackerel of identical origin and the fish flesh was digested with HCl on the steam bath for 90 min. One portion of this digested material was then extracted with ethyl ether in the manner previously described.¹ The second portion was then extracted by a mixture of ether and petroleum ether as described in the last report.²

The two quantities of extract were quite similar in appearance although the ethyl ether extract was slightly darker. Various constants of the two portions of the extracts were then determined (see Table 1).

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

¹ *This Journal*, 29, 46, 1946.

² *Ibid.*, 31, 334, 1948.

TABLE 1.—Constants of crude fat (extracts) from canned mackerel

DETERMINATION	WT. OF FAT USED	ETHYL ETHER EXTRACT			MIXTURE OF ETHYL ETHER AND PETROLEUM ETHER EXTRACT		
		SUB 1	SUB 2	AVL.	SUB 1	SUB 2	AVL.
Saponification Number (Methods of Analysis, 1945, 497, 31.24)	5 g	187.6	—	187.6	189.1	—	189.1
Unsaturation Residue (Methods of Analysis, 1945, 506, 31.43, 1st paragraph)	5 g	0.61 %	—	0.61 %	0.61 %	—	0.61 %
Lipoid Phosphoric Acid (Methods of Analysis, 1945, 348, 23.12(b))	Approx. 1 g	0.045 % 0.51 %	0.048 % 0.55 %	0.047 % 0.53 %	0.025 % 0.28 %	0.029 % 0.33 %	0.027 % 0.31 %
As P_2O_5	Approx. 1 g	140.8	141.5	141.1	120.1	119.1	119.6
As Lecithin	Approx. 10 g						
Hanus Iodine Number (Methods of Analysis, 1945, 494, 31.18)							
Free Fatty Acids (Method: 25 ml neutral eth- anol added, heated almost to boiling, titrated with 0.1 N NaOH to phenolphthalein)		18.0	—	18.0	18.6	—	18.6
Acid Number		9.1 %	—	9.1 %	9.4 %	—	9.4 %
As Oleic Acid		3.8*	—	3.8			
Free Fatty Acids* (Method as above, fat ex- tracted by ethyl ether in continuous extrac- tor, no digestion.) Acid Number	Approx. 5 g						

* This value obtained in order to determine amount of fatty acids due to acid digestion.

DISCUSSION

The additional work on the ether extract of canned mackerel shows that the composition of this crude fat is normal and involves no unusual problems.

The fish fat obtained by extracting with ethyl ether alone and with a mixture of ethyl ether and petroleum ether are identical for all practical purposes.

REPORT ON SPICES AND CONDIMENTS

By SAMUEL ALFEND (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.*), *Referee*

The Referee has studied the recommendations of the Committee on Classification of Methods, and if the suggested changes are adopted by the Association at this meeting, he expects to submit recommendations in 1949 to bring the chapter into conformity with the new classification.

VINEGAR

Associate Referee Loughrey submitted the tentative permanganate oxidation number to collaborative study on a distilled vinegar, a fortified wine vinegar, and two colored dilute acetic acid solutions. The titrations obtained on the dilute acetic acid solutions were nil. Those on the distilled vinegar were fairly uniform at approximately 4 ml. The results on the fortified wine vinegar were very high and erratic. The method obviously is suitable for the purpose for which it is proposed—to distinguish dilute acetic acid solutions from distilled vinegar. It is believed that adoption of the method as official need not wait for the accumulation of data on the actual oxidation numbers of commercial distilled vinegars from various sources.

The Associate Referee made a comparative study of the official method for tartrates in vinegar and the bitartrate method for tartrates in fruits. The results indicate a clear advantage for the bitartrate method. The Referee agrees with the recommendation to continue the studies.

Loughrey has studied the various tests for caramel in vinegar, but is not yet ready to make a recommendation. The studies should be continued.

MAYONNAISE AND SALAD DRESSINGS

No report was submitted by the Associate Referee. The work suggested for this year should be continued.

MUSTARD

Associate Referee Garfield has developed a procedure for determination of sugars in prepared mustard. The recoveries are slightly too high. The studies he has outlined for next year should be carried out.

* Present address, Kansas City Station, Kansas City, Mo.

In studying the methods of Terry and Corwin for the pungent principles in different varieties of mustard seed, Garfield has found volatile oil in so-called "white" or "yellow" mustard seeds, contrary to the statements of the English workers. He has therefore taken steps to obtain authentic seeds of known botanical classification, and plans to work on these samples next year.

VOLATILE OIL IN SPICES

No report has been submitted by the Associate Referee for several years. The topic should be reassigned and studies should be continued.

RECOMMENDATIONS†

It is recommended—

(1) That studies on the application to vinegar of the Mathers test for caramel, and the test for caramel in wine (15.39) be continued.

(2) That the tentative permanganate oxidation number, with the slight changes described in the Associate Referee's report, be made official, first action).

(3) That methods for the quantitative determination of free mineral acids be further studied.

(4) That studies of the determination of tartaric acid and tartrates in vinegar be continued.

REPORT ON VINEGAR

By JAMES H. C. LOUGHREY (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

Subcommittee C, (*This Journal*, 31, 54, 1948), made the following recommendations for work on Vinegar:

(1) That the method for the determination of caramel in vinegar (33.78) be dropped and that studies be made of other tests for caramel, such as Mather's test and the test for caramel in wine (15.39), to determine their applicability to the detection of caramel in vinegar.

(2) That the permanganate oxidation method be applied to a number of samples of distilled vinegars of known history in order to ascertain if it has value in differentiating this type of vinegar from a dilute acetic acid.

(3) That the method for the quantitative determination of free mineral acids in vinegar be further studied.

(4) That studies of the determination of tartaric acid and tartrates in vinegar be continued.

RECOMMENDATION (1)

The Associate Referee has conducted tests on a number of vinegars, comparing the present tentative method (33.78) with Mather's test and

† For report of Subcommittee C and action of the Association, see *This Journal*, 32, 58 (1949).

with the confirmatory test for caramel in wines (15.39). Mather's test involves coprecipitation of the caramel with pectin from an acidified alcohol solution. The caramel is confirmed by the use of 2,4-dinitrophenylhydrazine, which reagent appears to offer several advantages over the present reagent, phenylhydrazine hydrochloride.

The results of this comparison are given in Table 1. The cider vinegars used were received from two New England firms which are deemed to be reliable and which guaranteed the authenticity of the samples.

TABLE 1.—*Comparative results*

VINEGAR	PRESENT TENTATIVE METHOD	MATHER'S TEST	A.O.A.C. CONFIRMATORY
New (1947) Cider	Positive	Doubtful	Negative
Old (2½ years) Cider	Negative	Negative	Negative
Old (3½ years) Cider	Positive	Negative	Negative
Old (2 years) Cider	Negative	Negative	Negative
New (1½ years) Cider	Positive	Negative	Negative
Cider-Distilled Vinegar (50-50; Cider Vinegar 2 years old)	Positive	Negative	Negative
Very light caramel solution	Positive	Positive	Positive

It will be noted that the present tentative method (33.78) gave positive results on four of the six cider vinegars tested, which confirms previous findings.

The two methods with which the tentative method was compared appear to be more reliable. However, both methods will need further study before a recommendation is made. Of these two methods, the A.O.A.C. confirmatory test, in which the caramel is coprecipitated with zinc hydroxide, appears easier in manipulation. However, it suffers from the handicap that only a small proportion of the caramel actually present is precipitated by the zinc hydroxide; on known solutions of caramel, about 15 per cent of the caramel present is recovered. The figure is quite constant.

RECOMMENDATION (2)

The Associate Referee was able to obtain one distilled vinegar of known history. This vinegar was made from formula No. 35A of the Bureau of Internal Revenue, which calls for 5 gallons of ethyl acetate (not less than 85 per cent ethyl acetate upon saponification) to be added to each 100 gallons of ethyl alcohol. This formula was made into vinegar by the "closed generator" process. As received, the acidity was 5.5 per cent as acetic.

This sample of distilled vinegar was colored with amaranth and caramel, to resemble wine vinegar, and was submitted to collaborators as Sample A for determination of the permanganate oxidation number. Also submitted

were dilute acetic acid, colored with caramel, as Sample B; dilute acetic acid colored with amaranth and caramel as Sample C; and a wine vinegar to which ethyl acetate and a small amount of amyl acetate were added (to increase the oxidation number) as Sample D.

The method was substantially the same as that submitted in 1947. A few changes, in line with recommendations of collaborators, were made. Details of the method are published in *This Journal*, 32, 102 (1949).

The number of ml of the 0.5 *N* sodium thiosulfate required by the blank, less the quantity used in the determination divided by two is the permanganate oxidation number of the vinegar. Report on basis of the adjusted vinegar (4% acid).

If the permanganate oxidation number is more than 15, repeat the determination, using 25 ml of the adjusted vinegar plus 25 ml H₂O. Repeat this reduction by half until the ml of potassium permanganate used is less than 15. Calculate permanganate oxidation number to basis of 50 ml of adjusted vinegar.

The results reported by the various collaborators are given in Table 2.

TABLE 2.—*Collaborative results*

PERMANGANATE OXIDATION NUMBER				
COLLABORATOR	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
1	4.24	0	0	44.55 (10 ml)
2	4.46	0	0	—
3	4.40	0	0	36.25 (10 ml)
4	3.92	0.10	0.20	—
5	3.82	0	0	38.40 (10 ml)
6	4.47	0	0	38.12 (10 ml)
7	4.43	0	0	53.30 (5 ml)
8	3.84	0.05	0.14	48.85 (10 ml)
Assoc. Ref.	4.39	0	0	45.98 (10 ml)

The average of all nine results on Sample A, the "known" distilled vinegar, was 4.22. The results on the two samples, B and C, of dilute acetic acid, are in line with what was to be expected. Sample D was prepared purposely to give high values, inasmuch as some vinegars, such as wine vinegar, may give high values.

The method is an empirical one and is affected by time, temperature, and most of all by the concentrations of the oxidizing and reducing substances present in the reaction mixture. For that reason, it is recommended that, if the oxidation number is higher than 15, the determination be repeated, using smaller quantities of the vinegar. Two collaborators used 25 ml samples of Sample D and obtained permanganate oxidation numbers of 30.72 and 33.64, respectively; these values were not included in the above table. Using 25 ml samples, collaborator No. 1 obtained a

value of 31.21 for Sample D; the Associate Referee obtained 31.05 by the same procedure. It is obvious, therefore, that the aliquot taken for this determination must be small enough to allow for an excess of permanganate.

The method is of value *only* in differentiating between true vinegars and dilute acetic acid. The actual oxidation number when the value is high is not pertinent.

The Associate Referee wishes to thank the following collaborators for their prompt assistance: Paul Somerville, of H. J. Heinz Company; G. A. Michael, of the Massachusetts Dept. of Public Health, Division of Food and Drugs; and C. G. Cunningham, F. M. Garfield, E. H. Grant, R. E. O'Neill, P. B. Rokita, and S. Shendleman, all of the Food and Drug Administration.

RECOMMENDATION (3)

No work was done this year on the quantitative determination of free mineral acids in vinegar.

RECOMMENDATION (4)

The present official method for tartaric acid and tartrates in vinegars (33.84) is the same method as that for wines (15.28). This method appears to offer several opportunities for error. The method calls for evaporation of the vinegar to a sirupy consistency to remove excess of acetic acid, determination of the residual acidity, and the addition of a weighed amount of tartaric acid proportional to this residual acidity. The Associate Referee has found it difficult to drive off all the acetic acid; even as many as three evaporations will require as high as 3.0 ml of *N* alkali per 100 ml of vinegar. This would call for the addition of as much as 225 mg of tartaric acid to be added in the present method, which is an obvious defect because most vinegars will contain far less than that amount of tartaric acid. The occlusion of acetic acid by vinegar solids is well known in the determination of total solids in vinegar.

On the other hand, the bitartrate method for tartaric acid in fruits (26.32, 26.33) appears to be less subject to error. The Associate Referee has made a few analyses comparing the two methods, and the results are given below in Table 3.

TABLE 3.—*Comparison of present and bitartrate methods*

SAMPLE	TARTARIC ACID—MG PER 100ML VINEGAR	
	WINE METHOD	BITARTRATE METHOD
Wine Vinegar A	46.5 (225 mg added)	99.3
Wine Vinegar B	77.1 (168.8 mg added)	105.5, 105.5
Distilled Vinegar	6.7, 6.7 (18.8 mg added)	4.7 4.7
Distilled Vinegar and Tartaric Acid (1 mg per ml)	28.5 (112.5 mg added)	88.2, 100.8

The figures in parenthesis under the wine method show the amount of tartaric acid it was found necessary to add, following the directions given in that method. In every case, the vinegars were evaporated three times to the consistency of a sirup. The amounts of tartaric acid reported have been corrected for the added tartaric acid.

It is to be noted that in the distilled vinegar containing added tartaric acid (100 mg per 100 ml) the amount recovered was only 28.5 mg. In another determination by the wine method on this "known," the total amount recovered was 25.5 mg, despite the fact that a total of 212.5 mg (100 mg plus 112.5 mg added following directions) were present.

The Associate Referee recommends*—

- (1) That further study be made of Mather's test and of the test for caramel in wine (15.39) to determine their applicability to vinegar.
- (2) That further study be made of the permanganate oxidation number, as applied to distilled vinegars of known history.
- (3) That the method for the quantitative determination of free mineral acids in vinegar be further studied.
- (4) That further study be made of the applicability to vinegars of the bitartrate method for total tartaric acid.

REPORT ON SUGAR, ASH, AND PUNGENT PRINCIPLES IN MUSTARD

By FREDERICK M. GARFIELD (Food and Drug Administration,
Federal Security Agency, St. Louis, Mo.), *Associate Referee*

It was recommended by the Referee¹ and approved by Subcommittee C² and the Association that studies be made of a suitable method for the determination of sugars in prepared mustard. There appeared to be a real need for such a method to supplement the one for starch in prepared mustard. Previously, sugars and starch were lumped together under copper reducing substances by direct inversion. (This method, 33.40, was dropped, final action.³)

Clarification of the prepared mustard was first studied. After addition of calcium carbonate to take care of free acidity, clarifying agents such as lead acetate, lead carbonate, and lead acetate and carbonate were tried. Flocculent precipitates formed which could be easily filtered. A combination of lead carbonate (in place of calcium carbonate) and normal lead acetate seemed to give the best clarification. Difficulty was encountered in removing the excess lead. Both anhydrous potassium oxalate and

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 58 (1949).

¹ *This Journal*, 31, 340 (1948).

² *Ibid.*, 31, 55 (1948).

³ *Ibid.*, 31, 108 (1948).

sodium carbonate were tried, but the resulting precipitated lead would not settle or filter. This was overcome by centrifuging the material. The clear supernatant liquor was then poured through a filter. Anhydrous potassium oxalate seemed to work best.

Reducing sugars before and after inversion were determined by the Munson-Walker procedure. The resulting cuprous oxide was somewhat off color, tending toward the brown. The copper content was then checked by titration with thiosulfate (34.41). The sugars calculated from both the weighed cuprous oxide and the titrated copper showed that the weighed results were slightly high, but probably not high enough to warrant the additional work required to titrate the copper. For tabulation of results see Table 1.

DISCUSSION OF RESULTS

The recoveries of added sucrose are somewhat high, but they are promising. Further work will be undertaken in an attempt to account for these high recoveries. A correction for error due to volume occupied by insoluble mustard and lead carbonate in the clarification procedure should account for about 2 per cent of the high results.

Apparently the presence of starch has no effect on the sugar recoveries.

The results obtained by titration of the cuprous oxide are lower than those obtained by weight. The differences are slight and the additional work required to titrate the copper may be unwarranted.

Further work appears to be in order to account for the 1 per cent of reducing substances before inversion. Two or three authentic prepared mustards will be put up and the ingredients and the final product will be assayed in order to find the source of the copper reducing material. Examination of a sufficient number of commercial prepared mustards should give data to establish a figure for the normal content of reducing sugars in prepared mustard; values higher than this demonstrating the presence of added sugars.

Some work will be carried out on the effect of vinegar on the inversion of sucrose in prepared mustard under normal storage conditions.

METHOD USED

Approximately 10 g of prepared mustard were weighed into a 200 ml volumetric flask and clarified by the addition of 2 g of lead carbonate and 1 ml of 30 per cent normal lead acetate. The contents were made to volume and filtered. The excess lead was precipitated by the addition of anhydrous potassium oxalate, the lead oxalate settled by centrifuging and the supernatant liquid poured through a filter. Reducing sugars before inversion were determined in an aliquot by 34.39. A second aliquot was inverted by 34.24(c) and reducing sugars were determined by 34.39. Sucrose was calculated by 34.30.

TABLE 1.—*Tabulation of results—ounces*

DETERMINATION	1	1A	2	2A	3	3A	4	4A
Wt. prepared mustard—g.	10.124	10.113	10.282	10.062	10.150	10.098	10.047	10.316
Starch added	—	—	—	—	0.40	0.40	0.40*	0.40*
Sucrose added	—	—	0.4000	0.4000	0.4000	0.4000	0.4000*	0.4000
Reducing sugars before inversion as invert—								
from weighed Cu_2O	0.115	0.116	0.115	0.127	0.116	0.119	0.110	0.119
by titration	0.110	0.108	0.112	0.118	0.112	0.114	0.106	0.114
Reducing sugars after inversion as invert—								
from weighed Cu_2O	0.108	0.110	0.562	0.560	0.560	0.566	0.554	0.573
by titration	0.113	0.107	0.553	0.553	0.550	0.553	0.545	0.557
Sucrose—								
from weighed Cu_2O	—	—	0.425	0.411	0.422	0.425	0.422	0.431
by titration	—	—	0.419	0.413	0.416	0.417	0.417	0.420
% Recovery—								
from weighed Cu_2O	—	—	106.3	102.8	105.5	106.3	105.5	107.8
by titration	—	—	104.8	103.3	104.0	104.3	104.3	105.0

* Starch gelatinized.

VOLATILE OIL AND OTHER PUNGENT PRINCIPLES IN MUSTARD

Work on this subject was initiated last year. A literature study was made on available methods. The most promising appeared to be one by Tercy and Corran in the "Analyst."¹

The present A.O.A.C. method 33.26 can be used to determine the pungent principle (allyl isothiocyanate, which is volatile with steam) in black or brown mustard. According to Tercy and Corran the pungent principle (p-hydroxybenzyl ester of isothiocyanic acid, which is not volatile with steam) in white or yellow mustard cannot be determined in the same manner. They propose methods that will determine either principal alone, or in mixtures of the two.

When, in early experiments, so-called white or yellow mustards gave allyl isothiocyanate by the A.O.A.C. procedure in contradiction to numerous literature references, work was stopped.

It was not possible for the Associate Referee to identify botanically the mustard seeds on hand or to have them identified in St. Louis. Efforts were then directed toward obtaining true black (*Brassica nigra*) and white (*Sinapis alba*) mustard seeds. Work will be continued when these seeds are obtained.

RECOMMENDATIONS*

It is recommended that this subject be continued.

No work was done on "Ash in Prepared Mustard." It is recommended that this subject be continued.

It is recommended that the method for "Starch in Prepared Mustard and Mustard Flour" (now official, first action) be made official, final action.

No report was given on volatile oil in spices, preparation of sample, or fat in mayonnaise and salad dressing.

REPORT ON METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

By H. J. WICHMANN (Food and Drug Administration, Federal
Security Agency, Washington 25, D. C.), *Referee*

CADMIUM

Previous work having demonstrated that certain grades of carbon tetrachloride will not produce accurate results for cadmium in the presence of 5 per cent alkali, the Associate Referee was faced with the necessity of

¹ *Analyst*, 64, 164 (1939).

* For report of Committee C and action of the Association, see *This Journal*, 32, 58 (1949).

either reducing the alkalinity of the sample solution or else especially treating the carbon tetrachloride before use. The first alternative might easily result in the incomplete removal of zinc, to which any dithizone cadmium method is particularly sensitive. Therefore, he adopted the other possibility and purified his carbon tetrachloride by treatment with hot sodium hydroxide solution and redistillation to remove the impurities which caused the disturbances, particularly the fading, later on in the determination. Certain brands of carbon tetrachloride or perhaps, certain deliveries of these brands, did not need purification, while others did. To be on the safe side purification may be required unless previous trial of the carbon tetrachloride with known quantities of cadmium demonstrates its purity. The Associate Referee used relatively large amounts of interfering metals, particularly zinc, in the collaborative samples to provide a most rigorous test of the method. Under ordinary circumstances samples would not contain so much zinc, but this metal is almost universally present in food or biological material and is in some instances extraordinarily high in quantity. The Referee has in mind the 400 p.p.m. reported in some maple syrups, the 1000–2000 p.p.m. in some oysters (wet basis), and 5000–10,000 p.p.m. (dry basis) in some plants grown on high zinc soils. The Associate Referee wished to devise a method that would take care of these exceptions as well as the usual run of samples and, therefore, introduced into it a double extraction with 5 per cent alkali. Apparently these precautions are necessary on samples containing micro amounts of cadmium and relatively large amounts of zinc, because all three collaborators obtained slightly high results on the sample with only 5 micrograms of cadmium.

The Referee believes these results are encouraging, and that collaboration should continue next year with the object of obtaining sufficient basis for inclusion in the seventh edition, *Methods of Analysis*.

LEAD

No work has been done on lead methods for a number of years because there seemed to be no necessity for it, the methods that had been developed appearing to be adequate. In the interim, what may perhaps be best described as improvements appeared in the literature. Before the publication of the 7th edition of *Methods of Analysis*, the Referee believes that the Association should have a report on these improvements, so that if found worthy they may be incorporated in the next revision. The Referee has in mind particularly the Bambach and Bourky method of separating bismuth and lead at pH 3.4, and the Synder extraction of lead at pH 11.5 instead of at 9.5. If this proposed change produces as good results as those at the lower pH, certain worthwhile advantages might accrue. The associate refereeship on lead has been allowed to lapse for the last several years, subject to the call of the Referee, who believes that this

refereeship should be resumed next year for a report on these improvements. Elaborate collaboration is not necessary perhaps to justify these proposed changes, which do no great violence to the principles of the lead dithizone method but rather seek to streamline them. A favorable report based on actual trial by an expert in the field may be all that is necessary to get the lead methods ready for promotion to official status in time for the next revision.

MERCURY

In the Referee's opinion the two most important items developed in connection with a mercury method in the last two years is the use of selenium to help in the oxidation of the organic matter and the new apparatus designed to effect complete oxidation of organic matter without loss of mercury even in samples containing a large proportion of water. The way is now cleared for the necessary collaboration leading to the adoption of a new mercury method to replace the one now in the Book of Methods, which has sometimes been described as an illustration of "wash-tub" chemistry. Such methods so aptly described may be condoned if we have nothing better, but when the chances for improvement are as good as at present, the Referee recommends that these new ideas be embodied in a method and subjected to immediate collaboration in time for revision.

DDT

The Associate Referee on the determination of DDT has prepared a set of directions for the sample preparation of most of the widely varying plant and animal material likely to contain this insecticide, followed by its determination by the two most promising methods tried last year. The Referee notes, however, that the Associate Referee is silent on the enzymatic method of sample preparation for fats and fatty materials. The Association should have a report by next year on the comparative efficiency of the enzymatic versus the acid method described by the Associate Referee. The same may be said of the purification of benzene extract of plant material containing large amounts of chlorophyll or carotene by absorption methods. No collaborative results were offered. The Referee strongly urges that such results are essential and should be on hand at the next meeting. Certainly the Association must include one or two methods for DDT, if not some of the other chlorinated insecticides, in the next revision of the Book of Methods. In order to do so the Referee urges that the Associate Referee start the collaborative work as soon after this meeting as possible.

No report has been received on the determination of DDT in canned foods. The Referee recommends that this subject be reassigned and that the next Associate Referee investigate the fate not only of DDT but of some of the other newer insecticides as well, in the canning process. If decomposition of these insecticides occurs inside the can, how does it

affect the methods used for their determination? This Association should have the facts.

BENZENE HEXACHLORIDE (BHC)

The determination of benzene hexachloride residues is in an unsatisfactory state. The infra red, chromatographic, or biological methods for the determination of the gamma or other isomers in the commercial insecticide seem to be well advanced, but these methods are impracticable for the determination of residues. The chlorine methods with their non-specificity defect are about the only ones that can be recommended at the present time for quantitative determinations. Colorimetric methods so far tried are based on the nitration followed by color production of three isomeric tri-chlor-benzenes and suffer from their volatility, which results in 5-15 per cent losses before or during nitration. In addition, interfering color production results from the other organic materials of the samples which require absorption or chromatographic separation. Hence there are low recoveries and compensatory errors. Much more work will be required to devise a satisfactory method along this line. The Referee understands that a method based on the rather specific absorption of the 1-2-4 trichlorobenzene at a particular wave length is a prospect. Whether it will be sensitive enough for spray residues is still to be determined.

The Referee's attention has been called by the Beechnut Company to the possibility that the disagreeable and characteristic odor of commercial benzene hexachloride can be made the basis for a qualitative test for such residues. When the evaporated benzene extract of benzene hexachloride residues are oxidized by nitrating mixtures and then made alkaline, the odor of the insecticide remains predominant, while most of the natural odors have been destroyed or have disappeared. Whether this idea can be worked into a good qualitative method suitable for all kinds of benzene hexachloride residues remains to be determined. It appears at least to have a considerable degree of usefulness.

A most sensational result from the use of benzene hexachloride in agricultural crops occurred in connection with the 1947 crop of potatoes. Benzene hexachloride mixed with soil to kill wire worms, or even sprayed on the growing plants, resulted in a large crop of fine looking U. S. No. 1 potatoes, but unfortunately they were unpalatable to consumers. No benzene hexachloride was found inside the potatoes by any method, qualitative or quantitative, now available. The unpleasant flavor of the potatoes therefore is believed to be the result of secondary reactions in the potato started by the benzene hexachloride. Other agricultural crops have been affected more or less in the same way. Potato crops produced in 1948 on the same soil have been likewise affected. It may be several years before the ill effects wear out. This unfortunate result will naturally curtail the use of benzene hexachloride on food crops and perhaps the energies de-

voted towards devising methods for determining this insecticide in spray residues can be turned into other directions.

CHLORDANE

One method for the detection or determination of chlordane in insecticides or residues has just been published in *Analytical Chemistry*. A paper describing another is on the program. It is too early to assess the possibilities of these methods or the demand for them just now. Appointment of a Referee may be postponed for a year to allow the methods to be shaken down to practical realities.

TOXAPHENÉ

The Referee knows of no method for the determination of this insecticide unless it is the chloride methods. We had some experience during the year with a chlorine-containing insecticide on cabbage that did not respond to tests for any of the other newer insecticides. Presumably it was toxaphene, but that fact could not be demonstrated. Some specific method for the determination of this insecticide in residues is needed, but there is no promise for one at this time.

PARATHION

Parathion is the name given to perhaps the newest insecticide to appear on the horizon in commercial quantities. It is an ethyl-p-nitro phenyl ester of thiophosphoric acid. It has great insecticidal activity, but unfortunately it also is very toxic to man and other warm-blooded animals. Apparently only small quantities are needed to protect a crop and its reasonable volatility is expected to result in low residues. It has been used only in limited commercial quantity on the 1948 crop but much greater usage is expected next year. It is thought to be the best insecticide developed to combat the mites that have increased so tremendously as the result of killing their enemies with DDT. A combination of DDT and parathion may therefore be expected in future spray residues.

Heretofore spray residues have been found mostly on the surface of fruits and vegetables, if the catalytic action (if it may be described as such) of benzene hexachloride on potatoes is omitted from consideration. In the case of parathion there is definite evidence that it may be transported through the root and stem system to the leaves and edible portions of plants. Besides evidence of translocation of parathion we believe it can also penetrate the skins of fruits or vegetables into their interiors. The factors of time, concentration, weathering, and volatilization that govern translocation or penetration have not yet been fully assessed but we undoubtedly have been presented with a new problem in sample preparation. The old method of "stripping" an insecticide from the surface of fruits or vegetables by acids or organic solvents may no longer be adequate.

Fortunately, a method for the determination of parathion was released by the manufacturer simultaneously with its experimental and commercial usage. The method is based on the reduction of the nitro group attached to the phenyl ring in the para position followed by diazotization and color production with another amine hydrochloride. The efficiency of the results has been in the neighborhood of 85 to 90 per cent. This accuracy has been good enough for experimentation and preliminary assessment of commercial residues. To us it is a challenge for further efforts for increased efficiency. Another method based on different principles for check purposes is also highly desirable. Therefore, the Referee believes the Association should appoint a new Associate Referee to carry forward the work designed to improve the method presented to us and get it ready for adoption and perhaps include it in the next revision of the Book of Methods.

COMPOUND 118

Your Referee has just learned of a new toxicant which has not yet been given a "manageable" chemical name; hence its provisionable numerical name. It is in about the same position that parathion was last year, *viz*, ready for experimental trial generally but not for commercial distribution. Its structure is said to be that of a double bridged naphthalene with six chlorine atoms confined to one of the phenyl rings. It is understood that work on a method for its determination is well on the way and possibly will be ready by the time material amounts of the toxicant become available. The Referee is pleased to see that manufacturers of new insecticides are giving more attention to methods for the determination of their products.

RECOMMENDATIONS*

It is recommended—

(1) That the study of methods for the determination of cadmium, copper, lead, mercury, and zinc in foods be continued, with collaboration where necessary.

(2) That the study on the determination of DDT in foods in general be continued with sufficient collaboration to support adoption and inclusion of a DDT method in the next edition of *Methods of Analysis*.

(3) That the effect of the canning process on the decomposition of the newer insecticides be studied, with respect to (1) the nature of possible decomposition products and (2) their effect on the methods of analysis of the insecticides.

(4) That a new Associate Referee on the determination of parathion be appointed for study of that subject.

* For report of Subcommittee C, and action of the Association, see *This Journal*, 32, 55 (1945).

REPORT ON CADMIUM

By A. K. KLEIN (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

The micro cadmium method described in *This Journal*, **28**, 257 (1945) was submitted for this year's collaborative study. In the 1947 report, the Associate Referee called attention to the fact that the purity of the carbon tetrachloride, used as solvent for the extraction reagent, dithizone, is of utmost importance. During the past year it was observed that certain reagent grades of the solvent require no further purification. A collaborator in the Pharmacology Division of the Food and Drug Administration obtained excellent cadmium results in the range 0-25 micrograms using reagent grades of carbon tetrachloride and dithizone, neither of which was subjected to any further purification. The uniformly good results were obtained even in the presence of 1000 micrograms of added zinc. The Associate Referee, however, sought to devise a procedure of purification which would insure a satisfactory quality of all supplies of the solvent. Subsequent good results were obtained when the solvent was first refluxed for 1-2 hours with 5% sodium hydroxide, washed thoroughly with water, dried with calcium chloride, filtered, and then distilled over calcium oxide. The Associate Referee then felt assured that collaborative study of the method was merited.

Each collaborator was supplied with two unknown samples marked I and II. They consisted of 25 grams of comminuted tomato pulp, previously tested and found to be free of cadmium, to which were added 5 and 20 micrograms of cadmium, respectively. In addition the following interfering metals were added as soluble salts.

	<i>Sample I</i> <i>Micrograms</i>	<i>Sample II</i> <i>Micrograms</i>
Cu	250	500
Pb	250	500
Bi	50	100
Hg	50	100
Zn	1000	2000
Co	50	100
Ni	50	100

A practice sample of the tomato pulp containing no cadmium was also submitted to familiarize the collaborators with the method. The same interfering metals were added as in the unknowns in the amounts such that 25 g, the recommended weight for practice, would contain 88 per cent of the amount added to sample II. Collaborators were instructed to use the entire contents of the containers of I and II.

Their results are as follows:

<i>Collaborator</i>	<i>Sample I mmg</i>	<i>Deviation mmg</i>	<i>Sample II mmg</i>	<i>Deviation mmg</i>
A	5.4	+0.4	19.6	-0.4
B	6.8	+1.8	20.7	+0.7
C	6.7	+1.7	19.0	-1.0

Collaborator A offered no comments in his report.

Collaborator B stated that the method appeared satisfactory. In trial runs with the practice sample he obtained a blank of 0.2 mmg, and 9.9 mmg and 19.6 mmg when 10 and 20 mmg, respectively, were added. He experienced some difficulty in matching the optical fields in the visual evaluation of the cadmium dithizonate. This difficulty is due, no doubt, to a deficiency of his photometer, for no other collaborator has spoken of any difficulty in the visual evaluation of the optical density. Another reason for believing that the instrument was at fault is that he subsequently made standards of 6.8 and 20.7 mmg cadmium and the optical densities agreed, as well as he could read them, with those of the unknowns.

Collaborator C stated that the method seemed straightforward but has the weakness of lack of stability of the dithizone-carbon tetrachloride reagent. This latter statement, while true, is not an inherent fault of the cadmium method, because all dilute dithizone solutions are somewhat unstable and for that reason must be freshly prepared.

Two other collaborators were supplied with samples. One has not yet reported his results other than to state that Beer's Law was not followed in the development of the standard curve past 15 micrograms. He was using untreated Merck Reagent grade carbon tetrachloride and was therefore advised by mail to follow the recommended procedure of purification. The last collaborator experienced such erratic recoveries in trial runs that it was obvious that he too was working with an impure reagent. He too was advised to follow the directions for purification.

As a whole the submitted results, when judged from the standpoint of absolute recoveries, with the exception of those of Collaborator A, are not so good as had been expected. However, when we consider the relatively large proportion of metals, the results are not disquieting. For instance, Sample I contained 5 micrograms of cadmium in the presence of 1700 micrograms of interfering metals, any one of which if not completely removed would read in part as cadmium. The same comment may be made for Sample II, which contained but 20 micrograms of cadmium in the presence of 3400 micrograms of interfering metals. Since such samples are not at all likely to be encountered in ordinary analysis, the method was probably submitted to a very rigorous test, especially as none of the collaborators had had previous experience with the method.

The Associate Referee, therefore, feels assured that further collaborative study is merited.

REPORT ON MERCURY

By A. K. KLEIN (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

The micro mercury method devised by Laug,¹ has been used by him and his associates in the successful determination of mercury in hundreds of samples of various biological materials and animals' diets. Prior to 1947 it had not been applied to determining residual mercury in apples. However, the use of phenyl mercuric ammonium lactate or acetate as a control measure for the fruit disease, apple scab, obviously requires a method for the quantitative estimation of small amounts of residual mercury. Some of the difficulties encountered in the application of Laug's method to apple analyses were described by F. M. Kunze.² In brief, erratic recoveries, always on the low side, were obtained unless selenium, either as the metal or as the selenite, was present during the preparation of the sample, by oxidation and digestion with concentrated nitric and sulfuric acids under reflux.

In the analyses of biological materials sufficient mercury was present so that an aliquot of the liquid digest could be used for the analysis, a dithizone method so manipulated that it is specific for mercury. Laug was well aware of the fact that the entire acid digest can not usually be used for the determination. The high acidity and the presence of oxidizing materials decompose the dithizone chloroform reagent so that untrustworthy results were obtained. When an aliquot of the digest was diluted as outlined in the written procedure, no decomposition of dithizone occurred.

The Associate Referee, however, was requested to establish the fact whether or not any mercury at all is present in apples which have never been subjected to any mercury treatment. The amounts of mercury found in this instance would be very small, if present even at all, and would require, therefore, the analysis of the entire apple digest in order to obtain a sufficient quantity of mercury which would not be confused with a small reagent blank. Accordingly, 50 g portions of whole apples were digested under reflux in the conventional manner in the presence of 0.1 g selenium as selenite. The resulting acidity was then 95 per cent neutralized with 50 per cent sodium hydroxide and then extracted with the dithizone reagent. Almost invariably the dithizone was decomposed so completely that no mercury would be extracted had any been present. Control runs with added known amounts of mercury to the apples prior to digestion, even in the presence of selenium, usually led to low recoveries because of this decomposition. Sulfites added to the digest after neutralization with alkali prevented decomposition of the dithizone but combined with the

¹ *This Journal*, 25, 399 (1942).

² *This Journal*, 31, 438 (1948).

mercury to form so stable an inorganic complex that no mercury at all could be extracted with dithizone. The final alternative was to digest the sample so completely that no oxidizing organic material would be left to destroy the dithizone. Since apples contain 80 to 90 per cent of water, so complete a digestion could not be obtained unless the water is removed during the digestion. As mercury salts are volatile when heated, a special apparatus was designed which would trap the water and condense any volatilized mercury compounds. This was accomplished by affixing a Soxhlet apparatus devoid of the syphon to the digestion flask. To condense the acid vapors from the digest a reflux condenser was affixed to the top of the modified Soxhlet unit. In this manner, the water and acid vapors liberated during the digestion collect in the Soxhlet unit. The resulting concentrated digest may then be digested as completely as necessary by the portionwise addition of nitric acid through a standard taper dropping funnel leading to the second vent of the digestion flask. When the digestion is finished, the liquid which collected in the Soxhlet unit is drained back into the digestion flask by opening the stop-cock. The entire solution is then refluxed in the usual manner and the vapors and condensate thus formed completely rinse the upper portions of the apparatus so that any mercury which had collected there is restored to the flask. In this manner a digest is obtained which does not decompose the dithizone reagent even though the entire sample be used. A preliminary survey of untreated apples indicates that very little, if any, mercury is present in the fruit.

In Laug's procedure a standard volume of dithizone reagent is added to the final solution in estimating the mercury and in preparing the working standard reference curve. Since the mercury dithizonate is developed in an acid solution, a very large proportion of the optical density is due to unreacted dithizone. This obviously restricts the range of mercury which can be measured by any of the usual photometers. Greenleaf surmounted this difficulty ingeniously in his copper method.³ Instead of adding a fixed volume of dithizone reagent, he added this reagent portionwise until only a slight excess was present. Then a volume of carbon tetrachloride was added to bring the total to a fixed amount. By measuring the optical densities of the resulting solution at wave lengths maximum for both copper and dithizone he was able to evaluate copper in larger amounts than if the conventional dithizone procedure had been employed. However, a preliminary application of Greenleaf's technique to mercury was not successful. In the presence of only a slight excess of dithizone the mercury complex was too sensitive to light. When the densities were evaluated by the neutral wedge photometer, the color shifted so rapidly that no accurate optical density could be evaluated. It is quite likely that the Beckmann photometer, which employs a much less intense light source, will overcome this defect.

³ *This Journal*, 30, 144 (1947).

The Associate Referee therefore feels justified in submitting the modified method to collaborative study during the forthcoming year.

REPORT ON DDT AS SPRAY RESIDUE ON FOODS

By ROSCOE H. CARTER (Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.), *Associate Referee*

It is recommended that two methods be adopted as tentative for the determination of DDT residues in plant and animal materials, (1) the determination of the total organic chlorine content by the sodium and isopropanol method, and (2) the colorimetric determination based on the nitration of the compound and the development of a blue color by the use of sodium methylate.

Method 1 is applicable to cases where the spray history of the material is known. It is simpler and requires less equipment than Method 2. It is not specific, since other organic halogen compounds are determined by the same procedure. Method 2 is quite specific and should be relied on for determinations where only very small amounts are present and as a confirmatory test for the presence of DDT. Since the compound known as TDE (or DDD) gives the same colorimetric reaction, the history of the samples is of importance.

Determination of "blank" values on untreated materials from the same source is of extreme importance, especially when only small amounts of DDT are present.

SAMPLE PREPARATION

The first step in preparing the sample is to extract or strip the material with an organic solvent such as benzene, pentane, or petroleum ether. An apparatus similar to the churn-type washer described by Fahey, Cassil, and Rusk (1) is very useful for stripping operations on apples, pears, leafy vegetables, forage crops, and similar materials which are firm enough to withstand tumbling for short periods of time. Extraction by soaking, with occasional shaking, is satisfactory for some types of material; extractions in Soxhlet apparatus may also be employed. Bulky wet materials, such as forage crops and leafy vegetables such as cabbage and lettuce, must be dried before extraction. Drying at 70° to 80°C. in a circulating air drier has been found very satisfactory. This prevents contamination of the solvent with water and allows a larger sample to be extracted. Care must be taken to prevent entrainment of inorganic chlorides.

Sample preparation for apples and pears and the precautions to be observed have been discussed by Wichmann *et al.* (2).

For animal fats, organs, and flesh samples the following method of preparation has been found satisfactory: A 100 g sample is macerated

with 100 ml of benzene. This mixture is transferred to an evaporating dish, and the benzene is evaporated on a steam bath. The residue is mixed with sufficient anhydrous sodium sulfate to form a crumbly mass, which is then dried in an oven at approximately 70°C and again extracted with benzene. Suitable aliquots of the benzene solution are taken, filtered if necessary, and analyzed by chlorine or colorimetric methods.

Milk samples are treated as follows: To a sample of 200 g or more an equal volume of 95 per cent ethyl alcohol is added, and the mixture is extracted with 250 ml of a mixed solvent (75 per cent of ethyl ether and 25 per cent of a hydrocarbon fraction with b.p. 60–70°C. (Skellysolve B) in a separatory funnel. After separation of the two layers, the aqueous phase is extracted again with three 100 ml. portions of the mixed solvent. The ethyl ether-Skellysolve B solutions containing the DDT and butter fat are then combined and suitable aliquots taken for chlorine or colorimetric determinations. Precautions must be taken here also to prevent entrainment of inorganic chlorides.

I. ORGANIC CHLORINE DETERMINATION (3,4,5)

REAGENTS

Concentrations are expressed as weight per unit volume throughout this paper.

Sodium.—Metallic, C. P.

Silver nitrate soln.—0.1 *N* or other suitable concentration.

Nitric acid.—Concentrated, C. P.

Isopropanol.—99 per cent, C. P.

Ammonium or potassium thiocyanate.—0.1 *N* or other suitable concentration.

PROCEDURE

(Applicable to strip solutions from fruits, vegetables, and forage crops)

Evaporate aliquots of the strip soln from fruits, vegetables, or forage crops on steam bath until most of solvent is removed. It is not desirable to evaporate to complete dryness, as DDT may decompose with loss of hydrochloric acid. Add 25–50 ml of 99% isopropanol, then add 2.5 g of metallic sodium in the form of ribbon or cut in small pieces, and shake flask to mix sample with isopropanol. Connect to reflux condenser and boil gently for at least $\frac{1}{2}$ hour. Shake flask occasionally. Eliminate excess metallic sodium by cautiously adding 10 ml of 50% isopropanol thru the condenser at the rate of 1–2 drops per second. Boil for an additional 10 min., and then add 100 ml of water.

Cool, add 2 or 3 drops of phenolphthalein soln, neutralize by adding nitric acid (1-1) dropwise, and then add 10 ml in excess. If the soln is colored, cool to room temp., transfer contents of flask and aqueous washings to small separatory funnel, and shake with 15 ml of a mixture of equal volumes of isoamyl alcohol and ethyl ether. Draw off aqueous layer into second separatory funnel and extract again with 15 ml of the isoamyl alcohol-ethyl ether mixture. Draw off aqueous layer into 250 ml beaker. Wash the two extracts successively with 10 ml of water, and repeat with second washing with another 10 ml of water. Combine aqueous wash solns with the aqueous soln in the beaker. The chlorine may then be determined by one of following methods:

(a) Add a slight excess of 0.1 *N* silver nitrate and coagulate precipitated silver chloride by digesting on steam bath for $\frac{1}{2}$ hour with frequent stirring. Cool, filter

thru a fast qualitative paper, and wash thoroly with distilled water. Add 5 ml of saturated ferric alum soln, and determine excess silver nitrate in filtrate by titration with 0.1 *N* potassium thiocyanate. Subtract the quantity of silver nitrate found in filtrate from that originally added. The difference will be that required to combine with chlorine in DDT. One ml of 0.1 *N* silver nitrate is equivalent to 0.0035457 g of chlorine. To obtain the amount of DDT multiply the chlorine value by 2.

(b) Add 0.1 *N* silver nitrate from a buret, in excess of amount necessary to precipitate all the chloride. Then add 5 ml of nitrobenzene and 0.5 g of ferric sulfate. Swirl flask to coagulate the precipitate. Back titrate excess silver nitrate with 0.1 *N* potassium thiocyanate until faint pink color appears. Cross-titrate with both standard solns, crossing the end point in each direction. (The end point, which is not too sharp, is more easily perceived in this way.) Calculate amount of DDT as in (a) from amount of silver nitrate required for titration.

(c) Cool the flask to room temp. and then transfer contents to 400-ml beaker. The volume of soln should be 200–350 ml. Titrate with standard silver nitrate using silver-silver chloride electrodes on an electrometric titrimeter. Calculate the amount of DDT as in (a).

(d) Make alkaline to phenolphthalein by addition of 1 *N* sodium hydroxide. Cool the flask and transfer contents to platinum dish. Evaporate to dryness and ignite as thoroly as possible at a temp. not exceeding dull redness. Extract with hot water, filter, and wash. Return the residue to the platinum dish and ignite to ash; dissolve in nitric acid (1+4), filter from any insoluble residue, wash thoroly, and add this soln to the water extract. Add 10% silver nitrate soln, avoiding more than slight excess. Heat to boiling, protect from light, and allow to stand until precipitate is coagulated. Filter on weighed Gooch crucible, previously heated to 140–150°, and wash with hot water, testing filtrate to prove excess of silver nitrate. Dry the silver chloride at 140–150°, cool, and weigh. Calculate the percentage of DDT from weight of silver chloride and weight of sample used.

NOTE (1): If original strip solution is highly colored, it may sometimes be cleared up by adding decolorizing carbon and filtering. Test decolorizing carbon for presence of chlorides by heating with dilute nitric acid (1+4), filtering, and adding silver nitrate soln to the filtrate. If chloride is present, wash with warm dilute nitric acid until the washings no longer give a positive test.

NOTE (2): If free sulfur is present, add 10 ml of 30% hydrogen peroxide to reaction mixture after it has been diluted with water immediately after refluxing, and heat to boiling for 10–15 min.

NOTE (3): If an electrometric titrimeter is to be used, removal of colors from solns is not important.

II. COLORIMETRIC DETERMINATIONS

REAGENTS

Nitrating acid.—A mixture of C. P. fuming nitric acid (sp. gr. 1.49–1.50) and C. P. concentrated sulfuric acid (sp. gr. 1.84), 1 to 1 by volume.

Sodium hydroxide soln.—2%.

Sodium chloride soln.—Distilled water saturated with C. P. sodium chloride. Technical salt is unsatisfactory because of dirt and colored impurities extractable by ether.

Cotton.—Extracted with acetone in a Soxhlet extractor, dried for several hours at 105°–110°C., and stored in a tightly-stoppered bottle.

Ether.—U.S.P. grade distilled before use. Ether that has been standing long enough to accumulate peroxides and aldehydes, or has been recovered after use in this method, is unsatisfactory, and should be purified before it is used again.

Benzene.—C. P., dry. It is conveniently dried by distilling thru a straight con-

denser until no more water distills over with the benzene, and then replacing the condenser with a dry one and continuing the distillation. Benzene that has been used in this method to dissolve the nitrated residues or to make dilutions thereof may be accumulated and recovered for reuse by distillation.

Sodium methylate soln.— $10.0 \pm 0.1\%$ of sodium methylate in dry C. P. methanol (10.0 g per 100 ml of soln). An excellent method of drying the methanol is to reflux with magnesium turnings (5–10 g per liter of methanol) and a small amount of iodine until the magnesium has completely dissolved, and then to distill with the exclusion of moisture. The soln is prepared by dissolving requisite amount of perfectly clean sodium or good grade of powdered sodium methylate (available commercially) in the dried methanol with cooling, using stirrer and reflux condenser protected by soda-lime tube. An aliquot of clear portion of this soln should be diluted with water and titrated with standard hydrochloric acid, phenolphthalein being used as the indicator. The concentration of the soln should be adjusted to $10.0 \pm 0.1\%$ by the addition of sodium or sodium methylate, or by dilution with dry methanol.

(The sodium methylate soln that is added to the benzene to develop color should be colorless and optically clear. If sediment does not settle completely on standing, soln should be filtered or centrifuged. Occasionally a turbidity or precipitate of crystalline material (probably sodium carbonate) will form when the centrifuged sodium methylate reagent is added to the benzene solns. This difficulty can be obviated largely by cooling standardized soln in refrigerator for day or two, centrifuging while cold, and decanting into another container.)

Sodium sulfate-sulfuric acid.—Dissolve 100 g of C. P. anhydrous sodium sulfate (oven-dried) in 1 liter of C. P. concd. sulfuric acid (sp. gr. 1.84) with aid of heat, and cool to room temp.

Fuming sulfuric acid-concentrated sulfuric acid.—A mixture of equal volumes of fuming sulfuric acid (20–30% sulfur trioxide) and concd. sulfuric acid (sp. gr. 1.84).

Sodium bicarbonate soln.—5%.

Technical acetone, technical chloroform, and petroleum ether boiling at 60° – 70° C. (Skellysolve B). These solvents should be redistilled before use.

PROCEDURE (6, 7)

(Applicable to strip solutions from fruits, vegetables and forage crops and extracts of animal products).

Evaporate aliquots of strip soln from fruits, vegetables, forage crops or extracts of animal products to dryness on steam bath. Remove last traces of solvent by using tube connected to a vacuum. If any water is present, add isopropanol and again evaporate to dryness.

Quantitatively wash residue from evaporation into a 500 ml separatory funnel with 150 ml of chloroform. For the analysis of butter or other fat, substitute for this residue a 5 g sample or an extract thereof from which the solvent has been removed. Place 100 ml of chloroform in second 500 ml separatory funnel, and extract chloroform solns successively with (1) 50 ml of sodium sulfate-sulfuric acid (concd), (2) 50 ml of sodium sulfate-sulfuric acid, (3) 50 ml of fuming sulfuric acid-concd sulfuric acid, and (4) 50 ml of sodium sulfate-sulfuric acid. (If this last wash is not light in color, it is advisable to use still another sodium sulfate-sulfuric acid wash.) Drain each acid wash (lower layer) from first funnel into second funnel and finally into a 250 ml cylinder. (The extraction in second funnel is used to minimize loss of DDT by slight emulsification of chloroform in acid washings. The funnels should be shaken vigorously each time and then allowed to stand for 10–15 min. before draining off acid layer. In the rare case where emulsion forms and does not

separate in 30 min., mixture may be centrifuged and poured gently back into separatory funnel. It is well to keep a small beaker under each funnel and to have a wet cloth handy to wipe any acid which may drip.)

After acid extractions are completed, filter chloroform from both funnels thru a 5 cm tightly packed plug of cotton in glass Gooch crucible holder into third 500 ml separatory funnel. Pipet off any chloroform that has risen to surface from combined acid washings in the cylinder, and run it thru the plug of cotton. Rinse the two funnels and the cotton with chloroform, using ca 50–100 ml. Add enough 5% sodium bicarbonate soln (about 40 ml) to combined chloroform filtrate so that it will remain alkaline when tested with litmus paper after vigorous shaking. After allowing ca 10 min. for reasonably clear separation, filter only chloroform layer thru 5 cm plug of tightly packed cotton in glass Gooch crucible holder into a 500 ml Erlenmeyer flask with standard joint. Wash sodium bicarbonate soln remaining in funnel with two successive 30 ml portions of chloroform, and run mixture thru cotton into Erlenmeyer flask. If filtrate is not clear, filter again.

Add a glass bead to Erlenmeyer flask and recover chloroform on steam bath, using an all-glass system, until only about 10 ml of soln are left. Wash this soln quantitatively into large test tube (25×200 mm or larger) with acetone, add glass bead, and cautiously evaporate solvent on steam bath, removing last traces by inserting tube connected to vacuum line.

Nitration of sample.—Cool test tube in beaker of cold water and with pipet add 2.0 or 5.0 ml of the nitrating acid. Immerse test tube one-third to one-half its length in steam bath and heat for 1 hour. Since nitrations of even small quantities of materials may sometimes be violent, safety precautions should be observed. If there is much extraneous material, it is advisable to place test tube in ice-cold water, add cooled nitrating acid, and warm the tube cautiously to prevent sudden or violent nitration. When initial reaction has subsided, tube may be heated at 100° with safety. After 1-hour nitration, cool test tube in beaker of cold water, add 25 ml of ice-cold distilled water, and mix by gentle swirling. This procedure stops nitration, and test tube may be left overnight if desired.

Extraction of nitrated product.—Rinse contents of the test tube quantitatively thru small funnel into 125 ml separatory funnel with ca 25 ml of water from wash bottle and 50 ml of ether. A small, irregularly shaped piece of glass placed in funnel used for transfer will prevent the glass bead from falling into separatory funnel. Shake vigorously for at least 1 min. After layers have separated clearly, draw off and discard the lower layer. Wash ether with 10 ml portions of 2% aqueous sodium hydroxide until the washings are alkaline; one washing may be sufficient. Then wash ether with two 10 ml portions of salt soln. The final salt wash should be drawn off as completely as possible. Pack 0.75-inch plug of cotton tightly in glass Gooch-crucible holder, moisten it with ether, and allow ether soln from separatory funnel to filter slowly into 125 ml Erlenmeyer flask. Rinse the separatory funnel with 50 ml of ether in 4 or 5 portions, passing this ether thru the cotton in the Gooch funnel. If salt crystallizes in neck of separatory funnel, press stopper funnel in place firmly with rotating motion to prevent leakage of ether. Add glass bead to Erlenmeyer flask, warm the flask on steam bath with a gentle swirling motion until the bead starts bouncing, and recover or evaporate the ether completely. While flask is still being heated, insert a glass tube connected to a source of vacuum two-thirds of the way into the flask for at least half a min.; then remove the flask and stopper it. The analysis may be interrupted at this point if desired.

(The whole extraction procedure must be done carefully to avoid any loss, such as ether sprayed from the separatory funnel when the stopcock is opened to release pressure or when the glass stopper is removed. This type of loss can be minimized

by allowing time for the ether to drain away from the stopcock or the stopper before performing these operations.)

Development of color.—At this stage there is a choice of procedures, depending on the amount of DDT expected, the amount of soln necessary for use in making the photometric measurements, and whether it is desired to have some soln left to repeat the photometric measurements.

Procedure 1.—Add an accurately measured amount of benzene (for example, 5.00 ml) to residue in Erlenmeyer flask and swirl gently until it is dissolved. Use volume of benzene at least equal to one-third the volume necessary for use in absorption cell or tube of photometer. With pipet add 2 volumes (10.00 ml for 5.00 ml of the benzene soln) of sodium methylate reagent to 1 volume of benzene soln. Swirl gently until soln is homogeneous, pour into absorption cell or tube of photometer, and prepare to make the most important measurements 15 min. after the sodium methylate reagent has been mixed with benzene. (This procedure should be used only when it is known that the amount of DDT is very low and in the range where the color developed will be suitable for direct measurement in the photometer. If there is a possibility that the color developed will be too dark for direct measurement, it is preferable to use Procedure 2 rather than add more benzene and sodium methylate to the colored soln to dilute it.)

Procedure 2.—Add a measured amount of benzene (for example, 25.00 ml) to the Erlenmeyer flask, and swirl gently until residue is dissolved. To an aliquot (for example, 5.00 ml) add twice its volume of sodium methylate reagent, mix thoroly by gentle swirling, and pour into absorption cell or tube. (In some cases it is possible to mix the solutions directly in the absorption cell or tube. If the color is too deep, a photometric measurement may be made to obtain a rough estimate.) Dilute part or all of remaining benzene soln to a more suitable volume before removing new aliquot for development of color. If color is too light for good photometric measurement, rinse pipet used for first transfer with benzene into the Erlenmeyer flask, evaporate all solvent on steam bath, swirling flask gently to start bead bouncing, and when all the benzene is evaporated remove last traces by inserting glass tube attached to source of vacuum. This residue in Erlenmeyer flask should now be treated as in Procedure 1.

APPLICATION OF THE METHOD

Photometric measurements.—Spectrophotometric or photometric measurements should be made at the most important wave lengths or with the most important filters as close as possible to 15 minutes after the sodium methylate solution has been mixed with the benzene. Measurements at other wave lengths or with other filters can be made just before or after the most significant readings have been taken.

Absorption cells or tubes should be stoppered tightly. Absorption cells usually have glass covers or stoppers, but if test tubes are used, as in many routine photometric measurements, rubber stoppers washed free of sulfur are preferable to cork stoppers, contact with which will turn the solution yellow. Since the solutions on which optical measurements are made are strongly alkaline, absorption cells constructed with alkali-resistant cement should be used. The solutions should be left in the cells no longer than is necessary to make photometric measurements, after which the cells should be cleaned immediately. Although it might be expected that the

alkaline solutions would attack and etch glass cells, no such difficulty has been experienced during several months of use.

In any application of the method it is important to run a blank analysis on a sample of the same type of material being analyzed which has not been treated with DDT. The results, in terms of DDT or extinction values (never in terms of per cent transmission), should be applied as corrections to the values obtained at each wave length or filter used in the analysis of the DDT-treated samples. If appropriate blanks are not run, the results of the analysis may be high. Blank analyses should be made by diluting the blank runs in the same manner as the DDT-treated samples, or the corrections should be calculated to the same weight of untreated material as used in the analysis of the treated material.

A standard curve should be constructed by carrying through the nitration procedure and subsequent operations amounts of 25, 50, 75, and 100 micrograms of technical DDT. The photometric readings plotted on semilogarithmic paper against micrograms of DDT should lie in a straight line.

NOTE.—Samples containing considerable amounts of animal fat or highly colored extracts of plant material sometimes require several treatments with the sulfuric acid-sodium sulfate reagent before treatment with the fuming sulfuric-concentrated sulfuric reagent. A longer time of treatment is also of value in many cases.

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REPORT ON COPPER IN FOODS

By G. H. BENDIX (Continental Can Company, Inc.,
Chicago, Ill.), *Associate Referee*

The last report (1) by this Associate Referee dealt with the laboratory appraisal of the three most promising all-dithizone methods (2, 3, 4) for the determination of copper in foods. The results presented at that time showed no significant differences between the Bendix-Grabenstetter, Morrison-Paige, or Greenleaf methods. The objections to the Bendix method (conversion to enol tautomer) and the Morrison method (lack of sensitivity) did not affect the results as might have been expected.

TABLE 1.—*Results of collaborative samples*
p.p.p. Copper

COLLABORATOR NO.	BENDIX-GRANSTEDT METHOD						GREENLEAF METHOD					
	SAMPLE A		SAMPLE B		SAMPLE C & A		SAMPLE A		SAMPLE B		SAMPLE C & A	
	DUPLICATES	AVE.	DUPLICATES	AVE.	DUPLICATES	AVE.	DUPLICATES	AVE.	DUPLICATES	AVE.	DUPLICATES	AVE.
1	1 12.9 13.5	13.2	16.4 17.3	16.8	41.0 39.0	40.0						
	2 13.6 14.0	13.8	15.5 14.9	15.2	45.0 43.5	44.3						
2	1 16.0 16.0	16.0	12.2 12.1	12.2	45.9 47.6	46.8	15.2 14.4	14.8	15.1 10.7	12.9	45.1 45.1	45.1
	2 15.9 15.7	15.8	11.4 11.1	11.3	46.9 45.3	46.1	14.9 14.5	14.7	9.8 10.5	10.2	46.6 47.3	47.0
3	1 17.5 16.5	17.0	14.4 14.4	14.4	50.1 48.1	49.1	15.7 15.2	15.5	14.0 13.1	13.6	44.1 43.6	43.9
	2 17.1 15.6	16.4	16.8 15.1	16.0	49.8 44.8	47.3	15.9 16.6	16.3	11.8 11.8	11.8	43.8 43.8	43.8
4	15.5 ¹ 15.6 15.7 15.7		15.3 ¹ 14.8 14.4 15.3		51.8 ¹ 54.7 51.8 52.8	52.1						
Average		15.4		12.9		48.0	15.3			12.1		45.0
Max. Deviation		2.2		3.2		8.0	1.0			1.9		2.1
Ave. Deviation		1.1		1.4		3.0	0.6			1.1		1.1

¹ Separate assays.

In accordance with the recommendations of the last report the "two-color" method of Greenleaf and the "one-color" method of Bendix and Grabenstetter were submitted to collaborative study. A modification of the Bendix method was introduced for this work. The extraction of copper was made at pH of 3.2 rather than at pH 2.3 in order to reduce the shaking time of the extraction from 10 to 4 minutes.

Sample A consisted of regular Ovaltine powdered and thoroughly mixed to insure homogeneity. Sample B consisted of commercially canned peas, dried, powdered, and homogenized. Sample C was a standard copper solution containing 1.036 mg copper per ml to be added to Sample A for recoveries. Samples A and B were to be oven dried before weighing out the suggested sample sizes. Determinations were to be made in duplicate aliquots from each of two separately ashed samples per the Bendix and Greenleaf methods. An aliquot of solution C equivalent to 30 p.p.m. copper was added to Sample A for recoveries.

DISCUSSION OF RESULTS

The results presented in Table 1 indicate that the Bendix and Greenleaf methods produce comparable results on the selected samples. The Bendix method showed an average deviation of 1.1 and 1.4 p.p.m. copper in Samples A and B, and a deviation of 3.0 p.p.m. in Sample C & A, or in the latter case a recovery of 109 per cent. It should be noted that an average deviation of 1.1 to 3.0 p.p.m. copper over a range of 15-50 p.p.m. copper on the basis of a $\frac{1}{10}$ aliquot on a 2 gram sample will be equivalent to an average deviation of 0.2 to 0.6 micrograms of copper.

The Greenleaf method, although more difficult to carry out, gave an average deviation of 0.6 and 1.1 p.p.m. copper on Samples A & B, and 1.1 p.p.m. copper on Sample C & A, amounting to 99 per cent recovery. In terms of micrograms as above, the Greenleaf method average deviation is equivalent to 0.12 and 0.22 micrograms of copper.

COLLABORATORS' COMMENTS

(1) Changes in laboratory personnel necessitated the use of an inexperienced operator for these analyses and subsequent difficulties with the Greenleaf method produced erratic results which were not reported.

(2) "With respect to the copper methods, the Greenleaf procedure is undoubtedly more elegant since it does avoid the difficulties inherent in one-color methods. The additional manipulations, however, probably cancel out any possible advantage. Our personal opinion is that the procedure earlier published by Greenleaf is a much more satisfactory method if a theoretically perfect method is required for referee work. For routine work and for all practical purposes, however, the loss of copper inherent in the single-color method is probably sufficiently reproducible in unknowns and standards to reduce errors from this source to insignificance."

(3) No difficulties were reported. Samples were ashed as per Greenleaf method.

(4) This collaborator experienced difficulties with the Greenleaf method and felt the results obtained were not suitable to report.

COLLABORATORS

W. C. Stammer—Continental Can Company, Inc., Chicago, Illinois
O. R. Alexander—American Can Company, Inc., Maywood, Illinois
C. A. Greenleaf—National Canners Association, Washington, D. C.
Richard Coleman—Q.M.C., Chicago, Illinois.

RECOMMENDATIONS*

It is recommended—

- (1) That the "two-color" method of Greenleaf be accepted as a referee method pending official adoption.
- (2) That the "single-color" method of Bendix be accepted as a routine method pending official adoption.
- (3) That the Bendix and Greenleaf methods be submitted to further collaborative study to reconcile some of the difficulties encountered in this work prior to accepting as official A.O.A.C. procedures.

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No report was given on zinc, or on DDT in canned foods.

A contributed paper was presented, entitled "Spectrophotometric Method for the Estimation of Chlordane" by Bernard Davidow.

REPORT ON OILS, FATS, AND WAXES

By J. FITELSON (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Referee*

Reports on unsaponifiable matter and on peanut oil have been submitted. Difficulties in technique prevented collaborative work on the chromatographic purification of the unsaponifiable matter but current studies indicate that slight modification in this procedure may produce a satisfactory method. The collaborative results on the modified Bellier method for peanut oil demonstrate the usefulness of this test as a sorting procedure.

There will be no formal report on antioxidants, although a considerable

* For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 55 (1949).

amount of preliminary work has been done. As a result of a questionnaire survey, it has been found that the antioxidants most commonly used in fats are nordihydroguaiaretic acid (N.D.G.A.), Propyl or Lauryl gallate, tocopherols and gum guaiac. Methods for the determination of N.D.G.A. in fats are now being studied.

RECOMMENDATIONS*

It is recommended—

(1) That the Modified Bellier test (31.47–31.48) with the change suggested by the Associate Referee be adopted as official, first action, and that further collaborative studies be conducted.

(2) That the chromatographic purification of the unsaponifiable matter be further studied.

(3) That methods for the determination of antioxidants in fats be investigated.

REPORT ON PEANUT OIL

By G. KIRSTEN (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Associate Referee*

Five samples of oil were submitted to collaborators for the detection of peanut oil by the Modified Bellier Test. Collaborators were instructed to make tests in duplicate as directed in the A.O.A.C. *Methods of Analysis* 6th Ed., 31.48, with the exception that the alcohol used in the preparation of the alcoholic potassium hydroxide 31.47(a) need not be purified if the solution was to be used within a few days. Collaborators were also requested to repeat the tests, if time permitted, using a pipetted one ml sample in place of the weighed sample. The following technique was recommended for measuring the one ml sample of oil: "A short Mohr pipette with a fairly large opening at the tip should be used. Measure the oil by draining to the mark, holding until the meniscus in the pipette stops rising and again draining to the mark."

Collaborators who were requested to report turbidity temperatures and whether the test for peanut oil was positive or negative, obtained the following results:

All collaborators reported positive tests for peanut oil on Samples 1, 2, 3, and 4. Collaborator 2 reported Sample No. 5 as doubtful since his results coincided with the borderline temperature specified in the method. The others reported negative tests on Sample 5.

Sample No. 1 consisted of straight peanut oil; No. 2 of 10 per cent peanut oil in olive oil; No. 3 of 20 per cent peanut oil in soya oil; No. 4 of 15 per cent peanut oil in No. 5; No. 5 was a commercial oil labeled to contain 90 per cent corn oil and 10 per cent olive oil.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 56 (1949).

TABLE 1.—*Weighed samples*
Turbidity Temperatures °C

COLLABORATOR	SAMPLE NO. 1	NO. 2	NO. 3	NO. 4	NO. 5
1	36.5	15	21.5	17	9
	37*	14.5*	20.5*	18.5*	9*
2	38	18	24	21	13
3	34.5	15.5	21.5	18.5	11.5
	35.0	15.5	21.5	18.5	11.5
4	36.9	16.0	24.2	20.2	10.5
	37.3	16.0	24.2	20.2	10.5
5	35.5	13.7	22.0	18.5	10.2
	35.8	13.8	22.2	18.7	10.3
6	36.4	15.5	23.0	19.5	10.2
	36.8	15.5	22.8	19.4	10.4
Average	36.5	15.6	22.4	19.3	10.8

* Probably best results according to collaborator.

The results reported, while showing considerable variation in some instances, are in good general agreement. The results on the measured 1 ml samples agree very closely with those on weighed samples for the same chemist, indicating that there is no advantage in the use of weighed samples.

In order to see whether the turbidity temperature could be used as a rough indication of the amount of peanut oil present in mixtures, a curve was prepared by plotting turbidity temperature against per cent peanut

TABLE 2.—*1 ml samples*
Turbidity temperatures °C

COLLABORATOR	SAMPLE NO. 1	NO. 2	NO. 3	NO. 4	NO. 5
2	38	18	24	22	13
3	35.0	15.5	22.0	19.0	11.5
	35.0	15.5	21.5	18.5	11.0
4	37.4	15.9	24.4	20.5	10.6
	37.3	16.0	24.6	20.8	10.5
6	37.0	15.5	22.6	19.8	10.2
	36.5	15.3	23.0	19.5	10.2

oil for mixtures of peanut oil with various proportions of corn, cottonseed and soya oils. Samples of corn and peanut oil containing from 0 to 100 per cent of peanut oil were prepared and the turbidity temperature for each sample determined. The same procedure was repeated with cottonseed and peanut oil, and with soya oil and peanut oil. The turbidity temperatures for the same concentration of peanut oil in the three different oils

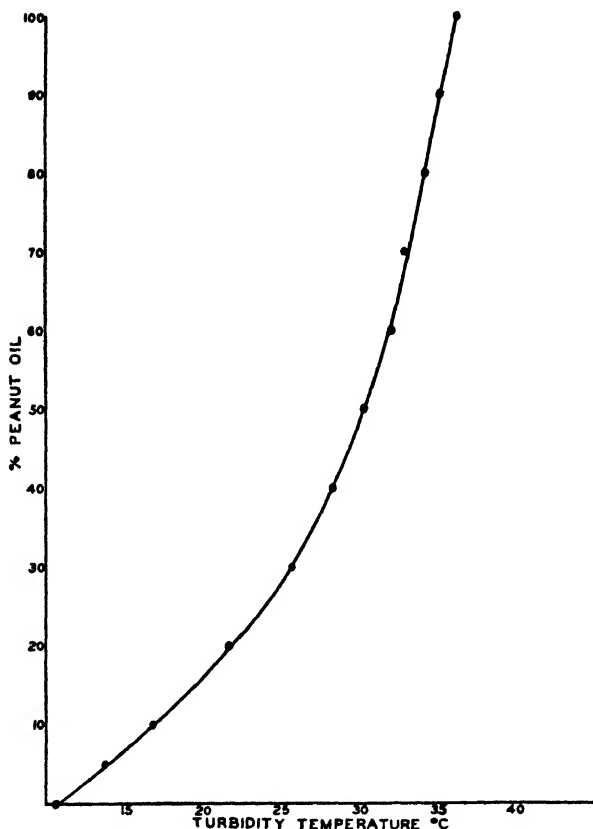


FIG. 1.—Average Turbidity Temperature—% Peanut Oil curve for mixtures of peanut oil with corn, cottonseed, and soya oils.

were averaged and the curve prepared by plotting the average turbidity temperature against per cent peanut oil.

Since the curve was prepared from average turbidity temperatures of different oils it can only be used to give a rough estimate of the amount of peanut oil present. However, since the turbidity temperatures for the mixtures containing the same concentration of peanut oil in the different oils did not vary more than the values obtained by different collaborators on the same sample, the preparation of separate curves did not appear to be justified.

Using the average curve and the average of turbidity temperatures reported by collaborators, the following results were obtained:

TABLE 3.—*Collaborative results*

SAMPLE	PEANUT OIL PRESENT	PEANUT OIL ESTIMATED FROM TURBIDITY TEMPERATURE
	<i>per cent</i>	<i>per cent</i>
1	100	100
2	10	7
3	20	22
4	15	15
5	0	0

While the individual turbidity temperatures do vary considerably, these results show that the test can be useful in obtaining an approximation of the amount of peanut oil present.

COLLABORATORS

The Associate Referee wishes to express his grateful appreciation to the following chemists who collaborated in this work:

A. B. Karasz, State of New York, Department of Agriculture and Markets.
George A. Michael, Department of Public Health, Boston, Mass.
H. P. Eiduson, Buffalo Station, Food and Drug Administration.
Frederick M. Garfield, St. Louis Station, Food and Drug Administration.
Sylvia Shendleman, New York Station, Food and Drug Administration.

RECOMMENDATIONS*

It is recommended—

(1) That the first sentence of 31.48, page 508, *Methods of Analysis*, be changed to read: "Weigh 0.92 g, or measure 1 ml of the oil into 125 ml Erlenmeyer flask with standard taper outer joint. If the oil is measured, use a short Mohr pipet with fairly large opening at tip, drain to lower mark, hold until meniscus stops rising in pipet and drain to mark again. Add 5 ml of the alcoholic KOH soln and heat for 5 min on steam bath, using air condenser to avoid loss of alcohol."

(2) That the modified Bellier Test, 31.47–31.48, *Methods of Analysis*, 6th Ed., be made official, first action.

(3) That further collaborative work be done on the method.

No report was given on antioxidants.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 56 (1949).

REPORT ON UNSAPONIFIABLE MATTER

By G. KIRSTEN (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Associate Referee*

Preliminary experiments showed the necessity for further study of the chromatographic purification of unsaponifiable matter before initiating collaborative work as recommended at the 1947 meeting.

The method using the chromatographic technique was published by Sylvester, Ainsworth, and Hughes¹ and consists essentially of the S.P.A.² extraction followed by purification of the unsaponifiable matter by passing the acidified ether extract through an alumina adsorption column to remove free fatty acids.

The publication reported good agreement between this method and the S.P.A. method. The Associate Referee has confirmed this agreement on several oils.

However, several technical details in this procedure required additional study. Thus, the specified amount of HCl was found to be insufficient in some instances, the method of drying the extract with anhydrous sodium sulfate is somewhat awkward, and some elution of the fatty acids occurs when ordinary reagent ether is used to wash the adsorption column, probably due to presence of alcohol. The specified quantity of HCl was found adequate if a preliminary water wash was used. Studies to improve the technic for drying the extract are continuing. No elution of fatty acids was observed if the column was washed with anhydrous ether or ether dried over calcium chloride.

Some preliminary results obtained using a sample of shark liver oil are given in the following table.

TABLE 1.—*Preliminary results*

METHOD	UNSAPONIFIABLE		TITRATABLE ACIDITY	
	(CORRECTED FOR ACIDITY)		(AS OLEIC ACID)	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1. S.P.A.	2.54	2.52	0.08	0.08
2. Chromatograph-reagent ether	2.50	2.52	0.15	0.16
3. Chromatograph-CaCl ₂ dried ether	2.51	2.67	0.06	0.06
4. Chromatograph-anhydrous ether	2.54	2.56	0.04	0.04

The chromatographic purification of unsaponifiable matter appears promising as a modification to shorten the S.P.A. method.

¹ *Analyst*, 70, 295 (1945).

² *Methods of Analysis*, A.O.A.C., 6th Ed., 31.40, p. 504.

It is recommended* that the chromatographic purification of unsaponifiable be further studied and be subjected to collaborative study.

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MACINTIRE (The University of Tennessee Agricultural Experiment Station, Knoxville), *Referee*

Because of changes in personnel among the Associate Referees, the findings upon soils and liming materials have been chiefly those reported currently by the Associate Referee on Liming Materials in a recommendation relative to the "2-point" barium hydroxide-barium acetate titration (as in (9) of the 1947 report, *This Journal*, 31, 43) and in a paper titled, "The Determination of the Sulfide Sulfur Content of Calcium Silicate Slags, in Relation to their Neutralization Value," as in (14) of the 1947 report, *Ibid*, 31, 44. Communications from the other Associate Referees express interest and willingness to pursue the severally assigned objectives.

RECOMMENDATIONS†

It is recommended—

- (1) That studies on the "combination dithizone-spectrographic method" and on the polarographic procedure for the determination of zinc in soils be continued.
- (2) That the study of the determination of copper in soils be continued.
- (3) That the utilization of carmin as an indicator in the determination of boron content of soils be studied further and that p-nitrobenzeneazo-1, 8-dihydroxy naphthalene-3, 6-disulphonic acid, or "chromotrope-B" be studied as a suitable reagent for the determination of boron in soils.
- (4) That further studies on pH in soils of arid and semi-arid regions be based upon soil systems of moisture content representative of an air-dry conditions.
- (5) That the analytical technic previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.
- (6) That a study be made as to the adequacy of calcium hydroxide as a fixative for fluorine in soil charges of 1 to 1 proportion with calcination at 500°C. in 5 to 60-minute periods.
- (7) That the direct distillation of unignited soil with sulfuric acid at 165°C, followed by distillation of an aliquot at 135°C, be studied collaboratively.
- (8) That the "2-point" barium hydroxide-barium acetate titration procedure for the determination of exchangeable hydrogen in soils, as re-

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 56 (1949).

† For report of Subcommittee A and action of the Association, see *This Journal*, 32, 46 (1949).

ported upon at this meeting, be studied further in relation to calcite equilibria in a variety of soils.

(9) That the survey and comparison of methods for the determination of phosphorus (a) that fraction in "available" state and (b) the proportions of organic-inorganic forms therein, be continued (*This Journal*, 30, 43).

(10) That the survey and comparisons of methods for the determination of exchangeable potassium in soils be continued. (*This Journal*, 30, 44).

(11) That the tentative procedures for neutralization value of calcium silicate slags, 3.11(a) be annotated by the statement "without correction for sulphide content." This constitutes a clarification of (13), 31, 44.

(12) That the procedure for the determination of sulfide sulfur content of calcium silicate slags as reported by the Associate Referee be adopted as tentative.

(13) That the Associate Refereeship on exchangeable calcium and magnesium be maintained.

REPORT ON EXCHANGEABLE HYDROGEN IN SOILS AND LIMING MATERIALS

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

In conformity with last years' recommendations, the Associate Referee made a study of the sulfide content in a large number of slags, and developed a method for its determination, as given in a paper submitted to the 1948 meeting of this Association.

The Associate Referee also made a study of titration procedures for exchangeable hydrogen in soils, utilizing ammonium acetate, calcium hydroxide, barium hydroxide and resultant values were compared with calcite decompositions in a number of soils under natural conditions of contact. The subject matter of this study is incorporated in a paper on Exchangeable Hydrogen of Soils presented to the 1948¹ meeting of the Association.

Upon basis of these two studies,

It is recommended*—

(1) That the procedure for sulfide sulfur in calcium silicate slags as given in the paper presented by the Associate Referee at the 1948¹ meeting, be adopted as tentative.

(2) That the "2-point" titration procedure for exchangeable hydrogen

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 46 (1949).

¹ *This Journal*, 31, 715 (1948).

in soils by means of barium hydroxide additions to 0.5 *M* barium-acetate soil suspensions as given in the paper presented by the Associate Referee be continued in relation to the soil-calcite equilibrium obtained under natural conditions of exposure.

REPORT ON EXCHANGEABLE POTASSIUM IN SOILS

By IVAN E. MILES (North Carolina Department of Agriculture, Raleigh, N. C.), *Associate Referee*

In continuation of the study of exchangeable potassium in soils, a further report is submitted this year, covering completed collaborative results to this date.

Detailed methods have been worked out in two laboratories for submission to each collaborator. Dr. Mehlich, of North Carolina State College, used a volumetric-ceric sulfate method, and Dr. Attoe and Prof. Truog of Wisconsin, a flame-photometric method. Collaborators were instructed to use any other preferred procedure, on condition that they use one of these methods as a basis of comparison.

The ceric-sulfate method is not new; and details of the Attoe-Truog method were published, under the title "Rapid Photometric Determination of Exchangeable Potassium," in *Soil Science Soc. Proceedings*, 11, 221-226 (1947).

Fourteen samples were submitted to each collaborator. The even numbers were check samples on the odd numbers. For instance, Nos. 1 and 2 were duplicate samples. So were all the other subsequent pairs through No. 14. Samples 1 and 2 were Cecil soil coming from the Piedmont area of North Carolina. Sample 3 through 8 were Norfolk soil from the coastal plain area of North Carolina. Samples 3 and 4 were untreated, 5 and 6 had one increment of potash added, and 7 and 8 had two increments of potash added. Sample 9 through 14 were Portsmouth soil from the coastal plain area of North Carolina. Samples 9 and 10 were untreated, 11 and 12 had one unit of potash added, and 13 and 14 had two units of potash added. This made a range of potash content from very low to relatively high, and involved both the Kaolinitic or 1:1 type of colloid in the Norfolk soils and the Montmorillonitic or 2:1 type of colloid in the Portsmouth soils.

These soils were air dried and mixed thoroughly, boxed, and labeled merely as 1 through 14, and sent to each collaborator. The results are given in Table 1. As will be seen from the table, the results agree better than in last year's collaborative work. However, there is still much variation in the extremes. Procedure A seems to have some advantage over Procedure B, as far as accuracy is concerned and is recommended for further study.

TABLE 1.—*Collaborative results of exchangeable potassium in soils*
All data reported as m.e. of potassium per 100 grams of soil

SAMPLE NUMBER	PROCEDURE A					PROCEDURE B			PROCEDURE C	PROCEDURE D	PROCEDURE E	PROCEDURE F		
	COLLABORATORS					COLLABORATORS			COLLABORATOR	COLLABORATOR	COLLABORATOR	COLLABORATORS		
	I	II	III	VI	AVG.	II	V	VI	AVG.	I	II	IV	VI	AVG.
1	0.958	0.890	0.950	0.878	0.919	0.890	0.936	0.985	0.937	0.931	0.920	0.904	0.920	0.908
2	.961	.870	.910	.894	.909	.880	.944	.960	.928	.916	.925	.870	.910	.910
3	.091	.074	.090	.100	.089	.080	.090	.090	.085	.091	.095	.110	.090	.096
4	.095	.083	.080	.102	.090	.085	.094	.090	.090	.092	.090	.104	.090	.095
5	.182	.170	.190	.194	.184	.172	.167	.177	.172	.161	.190	.182	.179	.179
6	.186	.176	.190	.189	.185	.164	.163	.177	.168	.156	.180	.184	.174	.177
7	.308	.300	.330	.298	.309	.302	.326	.298	.309	.320	.290	.289	.312	.302
8	.300	.308	.330	.298	.309	.302	.325	.300	.309	.307	.310	.295	.312	.305
9	.076	.064	.080	.080	.075	.070	.095	.077	.081	.080	.075	.095	.078	.085
10	.063	.071	.080	.070	.071	.070	.093	.070	.078	.079	.075	.094	.078	.084
11	.141	.148	.160	.141	.148	.150	.171	.147	.149	.145	.160	.160	.146	.147
12	.137	.143	.160	.145	.146	.140	.171	.151	.154	.155	.150	.166	.147	.151
13	.251	.275	.280	.247	.263	.257	.273	.250	.260	.235	.280	.270	.256	.257
14	.253	.267	.280	.247	.262	.250	.258	.253	.254	.243	.270	.258	.264	.258

Procedure A—Volumetric-Potassium cobaltinitrite-Ceric sulfate procedure. Details of procedure prepared by Mehlich.

Procedure B—Flame Photometric. Details of procedure by Allen and Truesdell, *Soil Sci. Soc. Proceedings*, 11, 221-226 (1947).

Procedure C—Flame Photometric-BaCl₂-triethanolamine. *Soil Sci. Soc. Proceedings*, 66, 429-445 (1943).

Procedure D—Volumetric. Extractions of potassium. *This Journal*, 16, pp. 137-194 (1933).

Procedure E—Volumetric—U.S.D.A. Circular 787, 1974.

Procedure F—Flame Photometric.—Extraction accomplished same as U.S.D.A. Circular 757, but amounts of potassium determined by use of Perkin-Elmer flame photometer.

The fine spirit of cooperation manifested on the part of collaborators and by the Association is appreciated.

COLLABORATORS

Brown, I. C., in Reitemeier's Laboratory, U.S.D.A.
 Holmes, R. S., in Reitemeier's Laboratory, U.S.D.A.
 Hallock, in Attoe and Troug's Laboratory, University of Wisconsin.
 Martin, J. C., University of California.
 Mehlich, A., North Carolina State College.
 Sterges, A. J., in MacIntire's Laboratory, University of Tennessee.
 York, E. T., Jr., in Peech's Laboratory, Cornell University, N. Y.

No reports were given on hydrogen-ion concentration of soils, boron and fluorine, zinc and copper, exchangeable calcium and magnesium, or phosphorus.

The contributed paper "A Volumetric Method for the Determination of Magnesium," by L. J. Hardin and W. H. MacIntire, was published in the preceding number, *This Journal*, page 139. Contributed paper entitled "The Determination of the Sulfide-sulfur Content of Calcium Silicate Slags in Relation to Their Neutralization Value," by W. M. Shaw, was published in *This Journal*, 31, 715 (1948).

REPORT ON ECONOMIC POISONS

By J. J. T. GRAHAM, (Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Department of Agriculture, Washington, D. C.), *Referee*

In planning the work on economic poisons for this year an effort was made to increase the number of projects to be studied. As a result of this effort the following chemists agreed to act as Associate Referees:

John W. Elmore, Bureau of Chemistry, State of California Department of Agriculture, Sacramento, California.

A. B. Heagy, Maryland Inspection and Regulatory Service, College Park, Maryland.

L. G. Keirstead, Agricultural Experiment Station, New Haven, Connecticut.

C. V. Bowen, E. E. Fleck, and S. A. Hall, Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Beltsville, Maryland.

F. A. Spurr, Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Department of Agriculture.

The Associate Referees have done a good job and their efforts are appreciated by the Association. Their reports speak for themselves and need no further comments or elaboration by the Referee.

In addition to the work of the Associate Referees, the Referee distributed two samples of pyrethrum powder to four collaborators for study of the Mercury Reduction method, the Seil method, and the Ripert

method. This work was in collaboration with the Consultative Committee on Insecticide Materials of Vegetable Origin, of the Imperial Institute, London. It was mentioned in the report to the Referee for 1947, and was undertaken in an attempt to obtain a uniform method for determination of pyrethrins, for world-wide use. The final results have not as yet been received, and a report for our Association on this work will be held over until next year.

Looking forward to next year, your Referee wishes to point out that there is an abundance of work that may be undertaken if enough chemists can be found who are willing to act as Associate Referees.

In the analysis of insecticides containing organic thiocyanates, the determination is usually based on a determination of nitrogen. It has been suggested that a more specific method should be studied.

Coal tar disinfectants have been used for a long time, and more recently the quaternary ammonium compounds have become very important in the disinfectant field. We have no official methods for analysis of these disinfectants and a study should be made of methods for their analysis as soon as possible.

The Referee has received a suggestion that a study be made of methods for determination of naphthalene in insecticides for control of lice on poultry; and of sabadilla alkaloids in preparations that contain ground sabadilla seed.

Other important economic poisons that offer an interesting field for study are aerosol insecticides, pyrethrins in presence of interfering substances, and products that contain chlordane, piperonyl butoxide, piperonyl cyclonene, or ferric dimethyl carbamate.

The Referee concurs in the recommendations of the Associate Referees as follows:

RECOMMENDATIONS*

It is recommended—

(1) That a study be made of the determination of rotenone in presence of other insecticidal or fungicidal ingredients or of diluents.

(2) That work be continued on the analysis of oil emulsions that are prepared with non-soap emulsifiers, giving consideration to the use of chromatography for determination of the oil.

(3) That the work on methods for analysis of "Antu" and "1080" be continued.

(4) That Methods No. 20 and No. 21 for determination of 2,4-Dichlorophenoxy acetic acid in herbicides be continued, giving consideration to the amount and manner of adding the indicator with a view to improving the end-point in the titration.

(5) That method No. 23 be further studied with emphasis placed upon a broader survey of ester mixtures and upon methods for breaking emulsions formed with this type of product.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 44 (1949).

(6) That the study of methods for analysis of tetraethyl pyrophosphate be continued.

(7) That the methods for determination of DDT based on the total chlorine, which were adopted as tentative methods in 1947, be adopted as official methods, first action.

(8) That the work on benzene hexachloride be continued, placing emphasis on determination of the gamma isomer in various formulations.

(9) That study be started on organic thiocyanates and dimethyl dithio carbamates.

REPORT ON RODENTICIDES

By JOHN W. ELMORE (Bureau of Chemistry, State of California, Department of Agriculture, Sacramento 14, California), *Associate Referee*

During the past year more specific methods have been sought for determination of "1080," (sodium fluoroacetate) and "Antu" (alpha naphthylthiourea.)

"ANTU"

Gibb's phenol reagent (1) and Folins's reagent (2) were found to give colors with dilute solutions of antu but reproducible results suitable for quantitative determinations were not obtained.

The method for determination of antu in rat poisons, suggested in last year's report (3) would be more specific if some further purification of the acetone extract were possible. It was found that aqueous alkaline solutions or suspensions of antu could be obtained which could be filtered. Precipitation of the antu with cuprous chloride then served further to isolate the material from accompanying contamination. Occasionally rat poisons are encountered containing acetone soluble nitrogenous material other than antu and in such cases this procedure may be applied as follows:

Obtain an acetone extract of the rat poison equivalent to 0.2 g antu, and evaporate all but 20 ml of the acetone. Add 100 ml 7% KOH (10 ml 1:1 diluted with water to 100 ml) and dilute to 300 ml with water. Filter on a Büchner funnel if a precipitate occurs. Add a few drops of methyl orange to the filtrate and make slightly acid with hydrochloric acid. Add 50 ml cuprous chloride soln (10 g CuCl, 50 g NaCl, 10 ml HCl, dilute to 400 ml with water). Filter on a small Büchner funnel and wash with water. Transfer the filter pad and precipitate to a 500 ml Kjeldahl flask and determine total nitrogen as usual (4).

NOTE—In carrying out this process some precipitation of antu occurs on acidification prior to addition of cuprous chloride. This is incomplete however, and the cuprous chloride must be added to obtain complete recovery.

"Antu" solutions in 95% ethyl alcohol were examined by spectrophotometer. A dip in the curve at 2210 Angstroms was the most significant feature found.

"1080"—Millimicrons against per cent transmission

Concentration: 2 mg. per ml. Solvent: Water

m μ	PER CENT TRANS.	m μ	PER CENT TRANS.	m μ	PER CENT TRANS.	m μ	PER CENT TRANS.	m μ	PER CENT TRANS.
200	54.3	4	93.0	8		2		6	97.0
1		5		9	97.6	3		7	
2	60.4	6	93.9	250	97.2	4		8	
3		7		1		5		9	
4	65.9	8	94.3	2		6	96.5	300	
5		9		3	97.1	7		1	97.2
6	71.2	230	95.2	4		8		2	
7		1		5		9		3	
8	75.8	2	96.0	6	96.7	280		4	
9		3	96.3	7		1	96.6	5	
210	79.9	4		8	96.6	2		6	97.3
1		5		9		3		7	
2	83.2	6	97.1	260		4		8	
3		7		1	96.1	5		9	
4	86.0	8		2		6	96.9	310	
5		9		3		7		1	97.4
6	88.0	240		4	96.1	8		2	
7		1	98.1	5		9		3	
8	89.6	2		6	96.0	290		4	
9		3		7		1	97.0	5	
220	90.9	4		8		2		6	97.5
1		5		9		3		7	
2	92.1	6	98.0	270		4		8	97.5
3		7		1	96.3	5		9	
								320	

RECOMMENDATIONS*

It is recommended that infra red absorption spectra of these compounds be examined for characteristics suitable for use in quantitative analysis.

REFERENCES

- (1) *Official and Tentative Methods of Analysis*, 6th Ed., page 321, 22.50 (c).
- (2) SNELL, *Colorimetric Methods of Analysis*, page 183.
- (3) ELMORE, J. W., *This Journal*, 31, 366 (1948).
- (4) *Official and Tentative Methods of Analysis*, 6th Ed., page 27, 2.25.
- (5) D. KRUGER and E. TSCHIRCH, *Chemical Abstracts*, Vol. 25, page 894.
- (6) DR. JOHN O. HUTCHENS and BEATRICE M. KASS, Private Communication.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 44 (1949).

REPORT ON DDT

By ELMER E. FLECK (Bureau of Entomology and Plant Quarantine, Beltsville, Md.), *Associate Referee*

It is recommended* that methods (1) through (6) as published in the Journal of the Association of Official Agricultural Chemists, 30, 64-66 (1947) and amended, *ibid.*, 31, 73 (1948), be adopted as official, first action.

It is also recommended* that the method for determining DDT in emulsions as published, *ibid.*, 31, 72 (1948), be adopted as official, first action.

REPORT ON TETRAETHYL PYROPHOSPHATE ANALYSIS

By S. A. HALL (Department of Agriculture, Bureau of Entomology and Plant Quarantine, Division of Insecticide Investigations, Beltsville, Md.), *Associate Referee*

Tetraethyl pyrophosphate is a colorless, odorless, high-boiling, hygroscopic liquid of extraordinary toxicity. It is the principal biologically active ingredient of so-called hexaethyl tetraphosphate, which is a mix-

TABLE 1.—*Chemical assays for tetraethyl pyrophosphate*
Comparative results in various laboratories (indicated by number) and different methods (M, V, H, and W)

SAMPLE	PER CENT TETRAETHYL PYROPHOSPHATE										AVERAGE
	1		2	3			4	5	6	U.S.D.A.	
	M	V	H	H	M	W	M	M	M	H	
A	99.1	93.8	93.5	—	—	—	97.6	98.4	98.5	97.9	97.0
B	38.7	39.4	39.8	36	38	36.8	35.9	40.0	36.6	36	37.7
C	38.7	37.3	35.6	33	38	35.4	34.2	36.5	35.9	35	36.0
D	8.1	11.0	10.8	10	7	7.4	7.0	8.3	7.4	8	8.5
E	34.8	35.4	31.5	33	35	32.9	34.2	35.5	34.9	33	34.0
F	42.1	41.4	39.0	38	43*	39.9	43.8	42.9	41.0	40	41.1
G	36.4	36.7	40.1	34	33	35.0	36.8	36.0	36.1	36	36.0

* Selected result. Assays on this sample ran 51, 21, and 43 per cent.

ture (1). When first produced in this country on a commercial scale, soon after the war, batches of hexaethyl tetraphosphate contained about 8 to 20 per cent of tetraethyl pyrophosphate; the insecticidal potency varied accordingly. Industry has in recent months shifted to a higher grade product, containing 34 to 41 per cent of tetraethyl pyrophosphate, together with relatively inactive ethyl phosphates and polyphosphates.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 45 (1949).

Analysis for tetraethyl pyrophosphate in the products of commerce is based upon selective hydrolysis of the sample followed by separation of the tetraethyl pyrophosphate from acidic constituents. The separated ester is most readily determined by completely hydrolyzing it to diethyl phosphoric acid, which may be readily determined by titration with standard alkali (2). Four separate analytical methods—all of them based upon the principle just described—were recently developed and applied by several collaborators to six samples of commercial products and a sample of purified tetraethyl pyrophosphate which served as a standard (3). Concordant results were obtained, as shown in Table 1, by the four methods, M, V, H, and W, which differ only in technics of separation or hydrolysis.

METHODS

Method M—Dvornikoff and Morrill (4)

APPARATUS

Dropper weighing bottle (5-ml) with ground-glass joint. Two 60-ml separatory funnels, pear-shaped, with stem cut off about $\frac{1}{4}$ inch below stopcock and ground at 45° angle. Erlenmeyer flask, 250 ml.

REAGENTS

- (1) Solution containing 9 g of sodium chloride per 100 g.
- (2) Benzene, nitration grade.
- (3) Sodium hydroxide, 0.5 *N*, and hydrochloric acid, 0.5 *N*, accurately standardized.
- (4) Bromothymol blue indicator soln, 0.1%, in water, solubilized as sodium salt.

PROCEDURE

1. Selective Hydrolysis

Place ca 3–5 ml of sample in weighing bottle, making the transfer with pipet or with the dropper itself. Avoid spilling material on ground-glass portion of the weighing bottle, and replace the dropper immediately to avoid moisture pick-up by the sample. Weigh the bottle and sample to the nearest milligram. Place 20 ml of 9% sodium chloride soln in a separatory funnel, marking this funnel "A" and the second funnel "B." Adjust the temperature of the salt soln to 30°C. by running warm or cold water over the surface of the funnel.

Add ca 1.0–1.5 g (for samples containing 50% of tetraethyl pyrophosphate or more) to 2.0–2.5 g (for samples containing less than 50% of tetraethyl pyrophosphate) of the sample to the salt soln in separatory funnel "A." Stopper the separatory funnel, shake until the soln is homogeneous, and let stand exactly 5 min. from the time the sample was added. Reweigh the weighing bottle to the nearest milligram to determine the exact sample weight.

2. Extraction

After five min. add 20 ml of benzene to the soln in separatory funnel "A" and shake vigorously for 30 seconds. Allow the layers to separate and draw off the aqueous (lower) layer of separatory funnel "B," taking care that none of the benzene enters the stopcock bore. Wash the benzene layer in "A" with 5 ml of 9% sodium

chloride by shaking for 10 seconds, separate the layers, and draw off the aqueous layer to funnel "B," this time allowing the benzene interface to pass just thru the stopcock bore.

Extract the combined aqueous layers in separatory funnel "B" with 10 ml of benzene for 10 seconds, separate the layers, and draw off and discard the aqueous (lower) layer, keeping the benzene interface above the stopcock bore. Wash the benzene in "B" with 5 ml of 9% sodium chloride by shaking for 10 seconds, separate the layers, and draw off and discard the aqueous layer, allowing the interface to pass just thru the stopcock bore. Drain the benzene layer in separatory funnel "B" into funnel "A."

Wash the combined benzene layers in funnel "A" with 20 ml of ice-cold, distilled water by shaking for 10 seconds. Separate the layers and drain the aqueous (lower) layer into a 250-ml Erlenmeyer flask, keeping the interface above the stopcock bore. Add 5-10 drops of bromothymol blue indicator soln and neutralize immediately with 0.5 *N* sodium hydroxide to the bromothymol blue end point, taking the first definite blue as the end point.¹

3. Assay

Run the benzene layer in funnel "A" into the cold-water wash previously neutralized in the Erlenmeyer flask. Rinse the walls of funnel "B" with 5 ml of benzene, drain into funnel "A," use this benzene to rinse the walls of funnel "A," and drain into the Erlenmeyer flask.

Add to the benzene-water mixture in the Erlenmeyer flask ca twice as much 0.5 *N* sodium hydroxide as is required for the expected amount of tetraethyl pyrophosphate (use 27 ml per gram of tetraethyl pyrophosphate expected), and stir for 1 hour vigorously enough to obtain good mixing of the two phases. A simple golf-club-type stirrer will give ample agitation. If desired, however, a shaking machine can be used with a ground-glass stoppered flask. After 1 hour, titrate to the bromothymol blue end point² with 0.5 *N* hydrochloric acid, swirling the flask to mix the benzene and water layers. Record the net volume of 0.5 *N* sodium hydroxide consumed (not including the volume required for the neutralization of the water wash.)

4. Calculations

Tests with pure tetraethyl pyrophosphate indicate that 97.8 per cent of the tetraethyl pyrophosphate present is recovered in the analysis. Of the remaining 2.2 per cent, 1.3 per cent is lost by hydrolysis during the 5 minute standing period before extraction, and 0.9 per cent remains unextracted. This loss of 2.2 per cent is taken into account in the following calculation:

$$\frac{\text{Net ml of 0.5 } N \text{ NaOH} \times 7.255}{\text{Wt. of sample} \times 0.978} = \% \text{ tetraethyl pyrophosphate}$$

or simplifying,

$$\frac{\text{Net ml of 0.5 } N \text{ NaOH} \times 7.42}{\text{Wt. of sample}} = \% \text{ tetraethyl pyrophosphate}$$

¹ Any diethyl acid phosphate present in the benzene soln is extracted by the cold-water wash and then neutralized. The end point should be reached as rapidly as possible consistent with accuracy. Some tetraethyl pyrophosphate is also extracted, but its rate of hydrolysis in cold water is so slow that only a negligible amount is hydrolysed and titrated. The unhydrolysed tetraethyl pyrophosphate in the water layer is not lost in the assay, since the water and benzene layers are recombined for the assay.

² The benzene layer will occlude droplets of water. It is therefore necessary to let the layers separate completely after each addition of standard acid or base when close to the end point. Since the first definite blue is taken as the end point, more accurate results are obtained by overtitrating with 0.5 *N* hydrochloric acid to a definite yellow and then titrating back to the blue end point with 0.5 *N* sodium hydroxide. The end point is sharp and stable.

Method V—Wreath and Zickefoose (5)

Weigh accurately ca 2.5 g of the material to be tested into 75 ml of 1-3 acetone-water mixture, temp. 25°C., and allow to stand 15 min. At the end of this time pour the soln containing the sample into a 250-ml Erlenmeyer flask containing 50 g of resin (Amberlite IR-4B) that has been washed with two 50-ml portions of the 1-3 acetone-water. Rinse the original flask quickly with 25 ml of 1-3 acetone-water and pour into the flask containing the sample and resin. Agitate for 5 min. Allow to stand for several seconds until the resin has settled, and then decant the liquid thru a coarse filter paper into a 500-ml volumetric flask. Give the resin five 30-second washes with 50 ml of 1-3 acetone-water, allowing the resin to settle after each wash before decanting the liquid into the flask. After the washing of the resin is complete, dilute to 500 ml with water and mix thoroly. This extraction procedure should take slightly less than one-half hour.

Pipet a 100-ml aliquot of the sample into a 400-ml beaker, add 50 ml of 0.1 *N* sodium hydroxide, stir, and allow to stand 1 hour at 50°C. This period of time allows complete hydrolysis of all tetraethyl pyrophosphate. After one hour cool to room temp. and back-titrate with 0.1 *N* hydrochloric acid to pH 6.0, using the glass electrode.

Calculations

$$\frac{500 \times \text{Net titration } 0.1 \text{ } N \text{ NaOH}}{\text{Sample wt.} \times 69} = \% \text{ tetraethyl pyrophosphate}$$

69.0 ml of 0.1 *N* sodium hydroxide is the calculated amount required for complete hydrolysis of 1 g of pure tetraethyl pyrophosphate.

This method has the following advantages:

1. The resin is an efficient agent in the separation of the acidic materials from tetraethyl pyrophosphate.
2. All extractions are run at room temperature. No ice water is required.
3. As solutions used are miscible with water in all proportions, no agitation is required in the hydrolysis stage of the procedure.
4. No factor is involved in the calculations, because tests show that less than 1.0% of the tetraethyl pyrophosphate present is hydrolyzed during the extraction.³

Method H—Hall and Jacobson (2)

With a small weighing buret weigh 2 g of sample to the nearest milligram into a dry 125-ml Erlenmeyer flask; stopper the flask. Remove the stopper to add ca 30 g of crushed ice. At this moment take note of the time or turn on an interval timer set for 60 min. Immediately after introducing the ice, drop in a strip of Congo red paper and, while swirling the flask, add rapidly from a buret or large medicine dropper a saturated soln of sodium bicarbonate. Place the flask in the refrigerator. Remove at the end of the 60-min. hydrolysis period and add at once 20 ml of chloroform. Mix by swirling the flask and then transfer the contents thru a funnel (to catch small ice crystals) into a 125-ml pear-shaped separatory funnel. Use ca 10 ml of chloroform to effect a quantitative transfer and to wash the ice crystals caught

³ The authors of Method V have recently modified their method by using a column of the acid absorbing resin. By suitable staggering of the samples in the improved method it is claimed that ten determinations may be run in 3½ hours.

in the funnel. When the lower chloroform layer becomes clear, in about 30 seconds, draw it off into a 250-ml Erlenmeyer flask. Do not allow any of the acidic aqueous layer to get to the bore of the stopcock. Then quickly extract (using only moderate shaking) the aqueous portion successively with two 10-ml portions of chloroform. Add two glass beads to the flask containing the combined extracts, place on the steam bath, and distill off the chloroform. Add 10 ml of ethyl alcohol, washing down the sides of the flask during the addition. Then insert a short glass tube (attached to a water aspirator or other source of vacuum) about half way into the flask to effect turbulence of the vapors while continuing the heating for about a minute longer. Remove the flask from the steam bath, add ca 100 ml of warm distilled water, washing down the sides of the flask. Cover the neck of the flask with a small inverted beaker and place in an oven at 50°C. ($\pm 2^\circ$) overnight (17 hours). In the morning titrate the accumulated samples with standard sodium hydroxide solution (0.1 to 0.15 *N*) using phenolphthalein indicator. No error is introduced if the samples are left in the 50°-oven for more than 17 hours.

Two moles of diethyl orthophosphoric acid (a strong monobasic acid) are formed for every mole of tetraethyl pyrophosphate, which has a molecular weight of 290.2. Per cent tetraethyl pyrophosphate may therefore be expressed as follows:

$$\frac{\text{Ml of NaOH} \times \frac{1}{2}N \times 290.2 \times 1.036 \text{ (correction factor)} \times 100}{\text{Wt. of sample in mg.}}$$

The correction factor 1.036 compensates for a loss of 2.0% in the selective hydrolysis and a further loss of 1.5% in the chloroform extraction of the aqueous solution of tetraethyl pyrophosphate. The equation reduces to:

$$\% \text{ Tetraethyl pyrophosphate} = \text{Ml of NaOH} \times N \times 15.04 / \text{Wt. of sample in grams}$$

NOTE: The results shown in Table 1 by the use of Method H are based upon its earlier and less refined form. As previously described above, the method has given results as follows at the Beltsville laboratory:

<i>Sample</i>	<i>% Tetraethyl Pyrophosphate</i>
A.....	97.9
B.....	36.3
C.....	35.0
D.....	7.4
E.....	33.9
F.....	40.2
G.....	36.4

Method W—Olson and Williams (6)

REAGENTS

Chloroform, technical grade; methyl orange indicator, modified with xylene cyanole F F; sodium hydroxide, 0.1 *N*.

APPARATUS

The apparatus listed below is in the most part standard laboratory equipment. For ease in handling, flasks and condensers with standard-taper ground joints are used.

Burette, 50 ml; condenser, Liebig; dropping bottle with ground-glass joint,

30-ml capacity, Erlenmeyer flask, 250 ml; glass beads; graduate, 100 ml; heater, Precision electric; separatory funnel, 250 ml with glass stopper, pear-shaped; Variac, type 200-C.

PROCEDURE

Weigh the dropping bottle after it has been filled about half full with tetraethyl pyrophosphate. With the dropper place 1.5–2 g of the sample in the Erlenmeyer flask, and reweigh the dropping bottle and contents. Record the weight to the nearest milligram. The difference between the initial and final weights is the weight of sample taken.

Add 20 ml of iced distilled water (0 to 5° C.) to the flask (note the time at which the water was added), swirl 5–10 seconds to insure soln, add 4–5 drops of the modified methyl orange indicator, and titrate immediately with 0.1 *N* sodium hydroxide. The first definite green color is taken as the end point. The amount of this titration is not important and need not be recorded. The color will probably change back to the red fairly quickly. However, no attempt should be made to obtain a permanent green color.

Allow the neutralized soln to stand 15 min. at room temp., counting time from the addition of the ice water. During this time clean the separatory funnel thoroly and grease the stopcock with stopcock grease. To prevent water from coming in contact with this stopcock grease any more than is necessary, pour 20 ml of chloroform into the separatory funnel.

After the soln has stood for 15 min., pour it into the separatory funnel on top of the chloroform. Rinse the flask three times using ca 5-ml portions of water, and add these rinsings to the soln in the funnel. Place the wet flask beneath the funnel.

Stopper the funnel and agitate its contents vigorously for 30 seconds. Release any pressure build-up by inverting the funnel and opening the stopcock carefully. Replace the funnel in its rack and allow the chloroform layer to settle. When two distinct layers have formed, draw off the lower layer into the Erlenmeyer flask, closing the stopcock when all but 1 or 2 ml of the chloroform layer has been withdrawn. Repeat the extraction procedure with three 15-ml portions of chloroform, leaving 1 or 2 ml of chloroform in the funnel each time when withdrawing the lower layer. Combine all the chloroform layers in the Erlenmeyer flask. After the last extraction discard the soln left in the separatory funnel.

Add 100 ml of distilled water to the chloroform extract in the flask and drop in 3 or 4 glass beads to prevent bumping. Place the flask on the electric heater and boil off the chloroform. When all the chloroform has evaporated, as evidenced by the presence of steam at the mouth of the flask, lower the condenser and reflux the solution for 2 hours. Adjust the Variac, controlling the heater voltage to about 80 volts so that only a mild boiling is obtained.

After this 2-hour reflux, rinse the condenser with about 20 ml of water and remove the flask. Cool the flask to room temp. by immersing it in an ice bath or by holding it under the cold-water tap. Add 4 or 5 drops of modified methyl orange indicator and titrate with 0.1 *N* sodium hydroxide to a definite green color.

Calculations

Calculate the tetraethyl pyrophosphate content from the following equation:

$$\frac{\text{Ml NaOH} \times N \text{ NaOH} \times 0.145 \times 1.025 \times 100}{\text{Wt. of sample in g}} = \% \text{ tetraethyl pyrophosphate}$$

$$\text{or} \quad \frac{\text{Ml NaOH} \times N \text{ NaOH} \times 14.86}{\text{Wt. of sample in g}} = \% \text{ tetraethyl pyrophosphate}$$

SUMMARY

Four methods for the determination of tetraethyl pyrophosphate in the products of commerce have been described. All methods are based upon the selective hydrolysis and separation of the tetraethyl pyrophosphate, which is then completely hydrolyzed to diethyl phosphoric acid and determined by titration.

The Associate Referee recommends* that studies to compare the different methods be continued so that the best procedure or modifications may be embodied in a tentative official method for collaborative testing.

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- (6) OLSON, R. A., and WILLIAMS, D., Westvaco Chemical Corp., So. Charleston, W. Va., *Private communication*.

REPORT ON 2,4-D HERBICIDES

By A. B. HEAGY (Maryland Inspection Service, Univ. of Maryland, College Park, Md.), *Associate Referee*

HISTORY

New methods for the estimation of 2,4-Dichlorophenoxy acetic acid, its salts, esters, and amides were considered desirable, in view of objections to the total chlorine methods previously in use. Among these objections are included inaccuracies arising from the presence of chlorides or other chlorine compounds, and the need for water-free samples when the sodium-alcohol method is used. The total chlorine procedure published in Soap & Sanitary Chemicals in October 1947 is open to the same objection that the presence of other chlorine materials will vitiate the results. Furthermore, any titration procedure must provide for elimination of all other organic acids to avoid high results.

Macro methods for the titration of 2,4-D and its compounds were first developed for use in the Bureau of Chemistry, Sacramento, California, and published in *Analytical Chemistry*, **19**, page 179, July 1947. In effect, the procedure and its modifications consist in titration of the acid groups when in the form of the free acid. In 2,4-D derivatives the samples are treated to produce the acid before titrating.

At the 1947 meeting the Association recommended that a study of these methods be made for the purpose of obtaining suitable procedures for the analysis of 2,4-D weed killers to be included in the chapter on Insecticides

* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 45 (1949).

and Fungicides, in A.O.A.C. *Methods of Analysis*. Copies of the procedures studied have been distributed for reference. It was also requested that collaborators examine the materials by the Parr Bomb method for the purpose of comparing results.

TABLE 1.—*Sample No. 1, Method No. 20*

FEDERAL AND STATE CONTROL LABORATORIES		
COLLABORATOR	RESULTS	OTHER METHODS
	<i>per cent</i>	<i>per cent</i>
A	50.78	
B	51.12	
C	50.69	37.58 ^{1,5}
D	51.58	46.13 ²
E	51.59	45.79 ²
F	52.38	
G	50.60	
H	50.13	
I	50.99 ³	50.59 ²
I	50.49 ⁴	50.76 ²
J	50.11 ²	50.70 ²
J	48.07 ⁴	
K	50.49	{ 50.37 ² 50.70 ²
L	53.90	
M	49.99	
N	47.95	
Average	50.68	48.78 ²
INDUSTRY LABORATORIES		
	<i>per cent</i>	
A	50.81	
B	50.92	
C	50.52	
D	50.28	
E	50.34	
F	50.27 ²	
F	49.83 ⁴	
G	50.25	49.88 ²
Average	50.40	
General Average	50.59	48.97 ²
High	53.90	
Low	47.95	

¹ Not included in average.

² Combustion Chamber Method.

³ Method #20 part 1.

⁴ Method #20 part 2.

⁵ Parr Bomb Method.

⁶ Corrected for inorganic chlorides.

TABLE 2.—*Sample No. 2, Method No. 21*

FEDERAL AND STATE CONTROL LABORATORIES		
COLLABORATOR	RESULT	OTHER METHODS
	<i>per cent</i>	<i>per cent</i>
A	91.81	
B	90.43	
C	91.44	91.69 ¹
D	91.11	90.95 ¹
E	91.04	91.12 ¹
F	95.08 ²	
G	93.50	
H	9.08 ³	
I	91.85	91.58 ¹
J	90.28	
K	91.68	{ 91.69 ² 92.65 ¹
L	96.70 ³	
M	91.36	
N	90.08	
Average	91.33	91.60 ¹
INDUSTRY LABORATORIES		
	<i>per cent</i>	
A	92.34	
B	—	
C	93.63	
D	92.29	
E	91.77	
F	96.44 ²	
G	91.38	91.67 ¹
Average	92.28	
General Average	91.62	91.63 ¹
High	96.70	
Low	9.08	

¹ Parr Bomb method.² Not included in average.³ Corrected for inorganic chlorides.

The Associate Referee in pursuing this work sent out to collaborators three samples of materials to be examined by these methods. Sample No. 1 was a 50% 2,4-D acid mixed with 50% talc, supplied by the J. T. Baker Chemical Company; No. 2 was a sodium salt of 2,4-D purchased from the consumer market; No. 3 was an isopropyl ester of 2,4-D supplied by the duPont Company.

Included in the list of collaborators are eight industry and sixteen federal and state laboratories. Most laboratories completed the work;

TABLE 3.—*Sample No. 3, Method No. 23*

FEDERAL AND STATE CONTROL LABORATORIES		
COLLABORATOR	RESULT	OTHER METHODS
	<i>per cent</i>	<i>per cent</i>
A	19.66 ¹	
B	48.04	
C	44.45	48.84 ²
D	45.87	49.63 ²
E	45.76	48.66 ²
F	45.80	
G	50.16	
H	1.34 ¹	
I	51.02	51.16 ²
I	51.60	51.76 ²
J	—	
K	48.91	50.05 ²
L	45.30	
M	25.24 ¹	
N	46.56	
Average	47.57	49.67 ²
INDUSTRY LABORATORIES		
A	47.27 ⁴	
A	47.45 ⁵	
B	—	
C	48.65	
D	47.65	
E	46.44	50.77 ⁶
E	—	51.33 ⁶
F	42.65 ¹	
G	48.94	51.77 ²
Average	47.73	51.27 ²
General Average	47.63	50.13 ²
High	51.60	
Low	1.34	

¹ Not included in average.² Parr Bomb Method.³ Combustion Furnace Method.⁴ Emulsions Present.

Emulsions Avoided.

⁵ Experimental Laboratory Method.

a few, however, examined only one or two of the samples.

Tables of results have been distributed from which a comparative picture of these methods can be drawn.

TABLE 4.—*2,4-Dichlorophenoxy acetic acid*
Commercial samples—Analyzed in Maryland Inspection Laboratory

2,4-DICHLOROPHENOXY ACETIC ACID			
SAMPLE NO.	GUARANTEE	TITRATION NO. 20 (2)	PARR BOMB
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	9.60	10.20	11.00
2	10.00	9.12	9.42
3	50.00	51.67	49.74
4	70.00	78.72	78.77

SALTS OF 2,4-DICHLOROPHENOXY ACETIC ACID			
		<i>Titration No. 21</i>	
1	14.00	14.58	16.71
2	14.00	14.98	15.06
3	14.00	15.02	15.40
4	77.00	81.42	75.90
5	20.00	21.16	21.73
6	19.80	21.18	21.57
7	2.00*	3.80	4.52

* Ethyl ester of 2,4-D (powder form).

A digest of the collaborators' comments is included to focus attention on those points of the methods requiring special care.

DISCUSSION

Results on samples No. 1 and No. 2 show a sufficient degree of consistency to prove the value of the procedures. Unquestionably, more familiarity with the manipulations involved would make for still closer agreement.

Sample No. 3 gave rise to the formation of very persistent emulsions, and caused general difficulty in applying the procedure. It was expected that results would vary widely. Most of the collaborators expressed the opinion that the method was unsatisfactory. Further investigation seems to be indicated here.

Results on all samples reported by the Parr Bomb method were somewhat erratic and not uniformly high, as expected. The inference can be drawn that incomplete combustion was an influencing factor.

Whether or not the end point in the titration is subject to further refinement is still to be learned. Since five collaborators reported it to be unsatisfactory it is possible that an optimum amount of the thymolphthalein indicator will effect an improvement. Further study will be recommended in this regard.

COLLABORATOR COMMENTS
Methods No. 20 & No. 21

FEDERAL AND STATE CONTROL LABORATORIES			
COLLABORATOR	AMT. OF INDICATOR	END POINT	NO. OF WASHINGS
A	0.5-1.0 ml	Unsatisfactory	3
B	5 drops 1 %	Satisfactory	5
C	5-7 drops	Satisfactory	3
D	1-2 ml 1 %	Unsatisfactory	3-5
E	1-2 ml 1 %	Unsatisfactory	3-5
F	0.5 ml	Satisfactory	4
G	1 ml .05 %	Satisfactory	3
H	10 drops	Satisfactory	4
I	8 drops	Satisfactory	3-4
J	5 drops	Satisfactory	3-4
K	10 drops	Unsatisfactory	3-4
L	1-2 ml	Satisfactory	3
M	5 drops	Satisfactory	3
N	4 drops	Satisfactory	10
INDUSTRY LABORATORIES			
A	12 drops	Satisfactory	3
B	1 ml .05 %	Satisfactory	3
C	18 drops 0.2 %	Unsatisfactory	3
D	4 drops	Satisfactory	3
E	6 drops 1 %	Unsatisfactory	3
F	20 drops	Satisfactory	3 (30 ml. ea.)
G	15 drops 0.1 %	Unsatisfactory	5

It is recommended*—

(1) That methods No. 20 and No. 21 be subjected to another year's study, with indicator added in 3 different quantities with the purpose of improving and standardizing the end point.

(2) That method No. 23 be subjected to another year's study with emphasis placed upon a broader survey of ester mixtures and upon methods of breaking the emulsions formed with this type of product.

LIST OF COLLABORATORS

INDUSTRY

J. M. F. Leaper, Chief Chemist, American Chemical Paint Company.
 E. C. Larsen, Asst. Director, Research Lab., J. T. Baker Company.
 A. W. Beshgetoor, Director, Main Lab., The Dow Chemical Company.
 J. F. Garrett, Process Section, E. I. duPont de Nemours & Company.
 W. R. Flach, Chief Chemist, Eastern States Farmers' Exchange.
 J. W. Zabor, Director of Development, Pittsburgh Coke & Chemical Company.
 R. W. Towne, Development Dept., Monsanto Chemical Company.

* For report of Subcommittee A and action of the Association, see *This Journal* 32, 44 (1949).

FEDERAL AND STATE OFFICIALS

Clemens Olsen, Arizona, Inspection Laboratory.
Herbert A. Rooney, California, Bureau of Chem. Dept. of Agriculture.
Charles V. Marshall, Canada, Dominion Dept. of Agriculture.
Lloyd G. Kierstead, Connecticut, Agricultural Experiment Station.
E. R. Winterle, Florida, Chemical Div., Agricultural Dept.
Giichi Fujimoto, Territory of Hawaii, Comms. of Agr. and Forestry.
A. C. Keith, Kansas, Control Div., Board of Agriculture.
John E. Schueler, Maryland, Inspection & Regulatory Service.
Albert B. Heagy, Maryland, Inspection & Regulatory Service.
Percy O'Meara, Michigan, Department of Agriculture.
Albert L. Weber, New York, Food & Drug Administration.
Howard Hammond, North Dakota, State Laboratories Dept.
T. L. Ogier, Texas, Agricultural Experiment Station.
Miss Edith Lawrence, Washington, Agricultural Experiment Station.
Mrs. Edith Huey, Washington, Agricultural Experiment Station.

Methods for the Collaborative Study of 2,4-D

Tentative No. 20

Determination of 2,4-Dichlorophenoxyacetic Acid

(1) Transfer 1 g of 2,4-D acid to 250 ml Erlenmeyer flask. Dissolve in 75 ml neutral ethyl alcohol and titrate with 0.1 *N* sodium hydroxide, using thymolphthalein as an indicator. Calculate percentage of 2,4-D on basis that 1 ml of 0.1 *N* NaOH is equivalent to 0.0221 g of acid.

(2) If sample contains insoluble carriers, transfer a sample equivalent to 1 g of 2,4-D acid to 250 ml beaker, add 25 ml of 1 *N* sodium hydroxide, and 50 ml of water. Stir to dissolve the 2,4-D. Filter and wash any insoluble matter that may be present and transfer to 250 ml separatory funnel. Proceed as directed in tentative method No. 21, par. 1, beginning "Neutralize with 10% sulphuric acid."

Tentative No. 21

Determination of Salts of 2,4-Dichlorophenoxyacetic Acid

(1) Dissolve sample equivalent to ca 1 g of 2,4-D acid in 50 ml of water (filter and wash samples containing insoluble carriers), transfer to 250 ml separatory funnel, neutralize with 10% sulfuric acid, and add 10 ml excess acid.

(2) Extract aqueous phase twice with 75 ml portions of ether. Wash combined ether extracts free from mineral acid with 10 ml portions of water until washings remain alkaline with addition of one drop of 0.1 *N* sodium hydroxide and phenolphthalein (2-3 washings).

(3) Transfer ether soln to 400 ml beaker, rinsing separatory funnel with ether. Add 25 ml of water, a few boiling chips, and evaporate ether layer on steam bath.

(4) Dissolve the aqueous mixture in 100 ml of neutral ethyl alcohol and titrate with 0.1 *N* sodium hydroxide, using thymolphthalein as an indicator.

(5) Each ml of 0.1 *N* sodium hydroxide is equivalent to 0.0221 g of dichlorophenoxyacetic acid.

Tentative No. 23

Determination of Esters of 2,4-Dichlorophenoxyacetic Acid
in Presence of Soap, Acids, Alcohols and Oils

(1) Reflux sample of weight equivalent to 0.7 g of the ester with ca 1 g of potassium hydroxide and 90 ml of 95% ethyl alcohol for one hour in a 250 ml S/T

Erlenmeyer flask. Transfer contents of flask to 250 ml beaker, add 50 ml of water, and evaporate on steam bath to about 50 ml to remove the alcohol. Transfer aqueous soln to 250 ml separatory funnel and extract with 75 ml of petroleum ether to remove unsaponifiable oils.

(2) Draw off aqueous phase into a 200 ml volumetric flask, add a few drops of 1 % phenolphthalein soln and a few drops of 1 to 1 soln of hydrochloric acid, to disappearance of the pink color, and then add 1 to 1 ammonium hydroxide soln until slightly alkaline. Add sufficient water to give volume of about 150 ml. Add slowly sufficient 10 % barium chloride soln to precipitate the fatty acids, make to volume, shake, and filter. The soln must be alkaline after the addition of barium chloride; otherwise the 2,4-D will precipitate.

(3) Transfer 100 ml aliquot in 250 ml separatory funnel, and acidify with hydrochloric acid. Proceed as in tentative method No. 21, beginning "Extract the aqueous phase twice."

Total Chlorine by Parr Bomb Method

Mix thoroly 14 g of powdered sodium peroxide and 0.75 g of powdered potassium nitrate in dry fusion cup, then mix in 0.4 g of finely-powdered cane sugar, and finally 0.2-0.25 g of sample, also in the form of a fine powder. If a larger amount of sample is used decrease amount of sugar in accordance with the content of carbon. Finely-powdered benzoic acid may be used in place of the cane sugar. Thoro mixing is essential. Take care to avoid spontaneous ignition when the organic matter comes in contact with sodium peroxide. Assemble the bomb and ignite. Cool bomb, open, and note whether there has been complete combustion. Rinse cover with distilled water into 400 ml beaker, set the fusion cup on its side, cover, and add enough water to cover about two-thirds of cup. Gentle heating on steam bath may be necessary to start decomposition reaction. After complete decomposition, remove cup and rinse, and heat the soln for few minutes to insure complete removal of all peroxide. Acidify with conc. nitric acid, boil for several minutes, cool, and precipitate with a measured excess of 0.1 N silver nitrate. Cool again, filter, and wash. Determine excess silver nitrate by titration with standard thiocyanate. Calculate percentage of chlorine in sample, and convert to the 2,4-D compound present, according to one of factors given below.

NOTES: (1) An alternate, and more rapid titrating procedure, used with complete success in this laboratory, consists in adding the excess silver nitrate, stirring to induce flocculation, then adding 5 ml of nitrobenzene, and stirring until all silver chloride particles are coated with the nitrobenzene (vigorous stirring is necessary). Titration with the thiocyanate can be done immediately, with no filtering.

(2) To avoid violent spontaneous ignition when liquid samples are analyzed in this manner it is advisable to use a weighing bulb. A light, round glass bulb, 1-1.5 cm in diameter, and having a short capillary neck is weighed, charged with sample by alternate warming and cooling, carefully wiped, sealed in a flame, weighed, and imbedded in the fusion mixture. Other operations are the same as above.

FACTORS:

Cl→2,4-D (acid) = 3.1172

Cl→Sodium Salt of 2,4-D = 3.4268

Cl→Isopropyl ester of 2,4-D = 3.7086

OTHER COLLABORATOR COMMENTS

Dow Chemical Company:

Sample No. 1 was first analyzed according to the prescribed procedure, i.e., using three washes. Results were as follows: 50.96, 50.86, 50.95, and 50.90, for an average of 50.92. Then, it was analyzed by the procedure using six washes giving the

following results of 50.59 and 50.50. The water washes were titrated with 0.1 *N* NaOH giving the following titration: (1) 0.49 ml, (2) 0.11 ml, and the next four 0.10 ml. This indicated that after the third wash there was no reduction in the amount of NaOH to titrate the acid in the water washes.

Using a slight excess of mineral acid, (.5 ml sulfuric acid). Three washes were used and the 2,4-D acid found was 50.48%. The washes titrated as follows: first, 0.10 ml, and the second and third washes, 0.09 ml.

Conclusion: A solubility factor is involved and the method is quite empirical. Therefore, the number of washes should be closely standardized. The difference between the figures using 3 and 6 washes become serious in good manufacturing practice. A standard correction might be advantageous.

In reply to the question regarding the practicability of methods No. 20 and No. 21, 15 cooperators reported these procedures satisfactory with more experience by the operators. Other comments were as follows:

- (1) Ether extracts should not be combined until after washing.
- (2) Use of filter paper may cause the difficulty in getting check results.
- (3) Difficulty encountered in washing sample off the filter paper.
- (4) In part (2) solution heated 50–60°C. to facilitate solution, and advocated the use of phenolphthalein.

Method No. 23

Three cooperators found the method satisfactory, partially because they had previous experience with it, but had not encountered trouble with emulsions before. The remainder declared the procedure unsatisfactory because of the difficulty in breaking emulsions that were formed. Comments from collaborators were as follows:

- (1) Suggested addition of ethanol took 65 hours to break emulsions.
- (2) Used modified method under investigation in experimental laboratory.
- (3) No method found to break emulsions.
- (4) Method unreliable, questioned possibility of 2,4-D being held by Ba salt.
- (5) Added 10 ml isoamyl alcohol to first extraction.
- (6) One day to a week required to break emulsions.
- (7) Emulsions centrifuged.

The following suggestions for breaking and preventing emulsions were submitted:

(1) Transfer the alcoholic solution after saponification and cooling to a 250 ml separatory funnel with 80 ml of water and extract with 75 ml of petroleum ether. Draw off the alcohol-water phase into another 250 ml separatory funnel, extract with another 75 ml portion of petroleum ether. Draw off the alcohol-water phase, wash the petroleum ether a few times with 10 ml portions of water, add the washings to the alcohol-water solution and evaporate down to about 50 ml on a steam bath. Make the residue to about 100 ml with water, cool the solution, and transfer to a 200 ml volumetric flask. After making to 150 ml with water, follow the remainder of Method 23 (2) in the precipitation of fatty acids.

(2) After refluxing sample for one hour with potassium hydroxide, transfer the contents to a 600 ml beaker. Add 50 ml of water and evaporate to 25 ml, swirl to aid particles to redissolve. Add 25 ml of water and evaporate to 25 ml. Transfer the warm solution to a separatory funnel with the aid of warm water. Add 75 ml of petroleum ether. Shake well for one minute. Let stand for several minutes. Draw off the bottom yellowish layer leaving the ether and emulsions in the funnel. Wash the contents of the funnel with successive 10 ml portions of a 10% salt solution, each time making sure that the emulsion remains in the funnel. After the third washing the

emulsion will be completely broken. The combined salt washings are collected separately from the yellow layer first drawn off. Treat the first layer drawn off which will contain practically all the fatty acids with mineral acid and proceed with the barium chloride precipitation as described in Method No. 23. Treat the salt washings in identical fashion. Combine the two solutions, make to 200 ml. Using filter cell collect 100 ml of clear filtrate. Proceed from this point as described in Method No. 21.

REPORT ON INSECTICIDES CONTAINING DERRIS OR CUBÉ (ROTENONE DETERMINATION)

By F. A. SPURR (Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

In the collaborative work that preceded the adoption of the present official method for rotenone determination, only the application of the method to samples of powdered derris and cubé roots was studied. In practice, however, the method has been adapted for use with products consisting of derris or cubé root powder mixed with other insecticidal and fungicidal ingredients and diluents.

No difficulty has been experienced in applying a modification of the official method to products in which, in addition to the derris or cubé root powder, the usual diluents were the only other ingredients.

Sulfur interferes in the determination of rotenone by the official procedure, but it has been found that a modification of the procedure by which the rotenone and resins are separated from the sulfur by means of acetone, has given satisfactory results.

Other substances, such as organic thiocyanates, oils, and various wetting or spreading agents, have interfered to such an extent that reliable results could not be obtained.

This year it was our intention to study the extent of the interference caused by several of the ingredients that are frequently incorporated in rotenone-bearing insecticides, with a view to determining the modifications to the official method that might be necessary to overcome such interferences. However, because of the large amount of extra work that was required of the personnel of the Insecticide Division in connection with the registration of products under the new Federal Insecticide, Fungicide, and Rodenticide Act, we were unable to conduct any study of methods for rotenone determination.

RECOMMENDATIONS*

It is recommended that a study be made of the determination of rotenone in presence of other insecticidal or fungicidal ingredients and diluents.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 44 (1949).

REPORT ON OIL EMULSIONS

By L. G. KEIRSTEAD (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

The simplicity and proven usefulness of chromatography for the separation of organic materials led to an investigation of its possibilities for the quantitative estimation of petroleum oil in self-emulsifying oil mixes. The following technique seems to have merit:

REAGENTS

Benzene

Petroleum ether

Sodium sulphate (anhydrous)

Absorptive powdered magnesia No. 2642. (Westvaco Chlorine Products Company, Newark, California)

Hyflo Super-Cel

EQUIPMENT

Chromatograph tube inside diameter 2 cm, height above joint 20 cm, with standard taper fittings.

Fisher Filtrator or equivalent for applying suction and catching solvent.

PROCEDURE

Prepare column by first putting wad of cotton in bottom of tube, turning on suction, and adding mixture of powdered magnesia and Super-Cel (1+1 by weight) tamping firmly occasionally until height of absorbent reaches six to seven cm. Place a 1 cm layer of anhydrous sodium sulphate on top to take up traces of moisture.

Weigh about 2 gm of the self-emulsifying mix into small beaker containing short glass rod. Place a 100 ml beaker beneath the column and turn on suction pump. Decant as much as possible of the oil mix into the column and reweigh, obtaining sample weight by difference. When material is drawn into column wash thru with small increments of a mixture (1+4) of benzene and petroleum ether, continuing until 75 ml have been used. Continue suction until column is nearly dry. Remove the 100 ml beaker and evaporate in a warm place, such as the top of an oven, until the solvent is nearly gone—overnight is convenient. Heat on steam bath ca two hours, then in an oven at 100° for 20 min., cool in desiccator, and weigh.

TABLE 1.—*Recovery of Shell Superior oil after washing through column*

HEATING	SAMPLE A	SAMPLE B	AVERAGE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Overnight, top of oven	98.92	98.73	98.83
Plus 2 hrs. on steam bath and 20 min. at 100° in oven	98.65	98.40	98.53
Plus 16 hrs. at 100°	97.25	97.52	97.39
Plus 68.5 hrs. at 100°	95.36	95.81	95.59

TABLE 2.—*Loss of Shell Superior oil on heating*

HEATING	RECOVERY		AVERAGE RECOVERY
	SAMPLE A	SAMPLE B	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Overnight, top of oven	99.95	99.97	99.96
Plus 2 hrs. on steam bath and 20 min at 100° in oven	99.32	99.60	99.46
Plus 16 hrs. at 100°	98.47	98.73	98.60
Plus 68.5 hrs. at 100°	96.81	97.08	96.95

TABLE 3.—*Recovery of oil from mixture of 90% Shell
Superior oil and 10% Triton X-100*

PERCENTAGE RECOVERY	DEVIATION FROM AVERAGE
101.27	+0.63
103.50	+2.86
98.53	-2.11
100.59	-0.05
99.31	-1.33
Average 100.64	

TABLE 4.—*Recovery of oil from mixture of 90% Shell
Superior oil and 10% Igepal 300*

PERCENTAGE RECOVERY	DEVIATION FROM AVERAGE
103.50	-1.22
102.82	-1.90
105.20	+ .48
106.71	+1.99
105.35	+0.63
Average 104.72	

TABLE 5.—*Recovery of oil from commercial mixes*

COMMERCIAL MIX	OIL FOUND, PER CENT		AVERAGE
A	74.53	76.53	75.53
B	82.99	82.68	82.84
C	95.12	94.98	95.05
D	84.59	84.88	84.74
E	97.51	97.71	97.61

The recovery of Shell Superior oil from the column is given in Table 1, together with data showing the effect of continued heating. Table 2 shows the effect of continued heating on Shell Superior oil which had not been put through the column. If the oil may be considered to be anhydrous as received, there is a loss of about 0.5 per cent in evaporating the solvents according to the procedure outlined above. Approximately one per cent of the oil is retained by the column as two fluorescent bands. This material is not eluted by the benzene-petroleum ether mixture but is readily washed out with alcohol. It is a gummy substance. The benzene-petroleum ether soluble fraction is lighter in color than the original oil.

This method of oil determination was applied to (a) a mixture of 90 per cent Shell Superior oil and 10 per cent Triton X-100 and (b) a mixture of 90 per cent Shell Superior oil and 10 per cent Igepal 300. Recoveries are given in Tables 3 and 4, respectively. They were somewhat high for both mixtures.

The method was also applied to five different commercial mixes whose actual oil content was unknown. Results are given in Table 5.

All of the above experiments yielded oils that would not emulsify on vigorous shaking with water, proving that the column did separate the oils from the emulsifiers. The high recoveries of oil from the known mixtures shown in Tables 3 and 4 would appear to indicate that both the Triton X-100 and Igepal 300 contained small amounts of oil. An attempt to separate oil from Triton X-100 and Igepal 300 by passing 2 gram quantities of each of these materials through the column failed, however, as the recovered material emulsified with water.

CONCLUSIONS AND RECOMMENDATIONS*

The idea of using chromatography for the determination of oil in emulsifiable oil mixes has enough merit to warrant investigation. The problem now is to hit on an adsorbent or solvent or some combination thereof that will remove all the oil, including the gums, without taking the emulsifying agents with it. When this is accomplished, the dream of the analytical chemist of putting a sample in one end of an apparatus and collecting the desired component at the other will have been realized. A comparison with existing procedures will then be in order.

No report was given on benzene hexachloride.

No report was made on disinfectants or on leathers and tanning materials.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 44 (1949).

ANNOUNCEMENTS**Referee Assignments, Changes, and Appointments****MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS:**

Robert I. Tenney, Wahl-Henius Institute, 64 East Lake Street, Chicago, Ill., has been appointed Associate Referee.

ECONOMIC POISONS:

Fred I. Edwards, Bureau of Insecticide Investigation, Bureau of Entomology and Plant Quarantine, U. S. Dept. of Agriculture, Beltsville, Md. has been appointed Associate Referee on Parathion.

FEEDING STUFFS:

Richart T. Merwin, Agricultural Experiment Station, New Haven, Conn., has been appointed Associate Referee on Sulfa Drugs in Feeds.

SOILS AND LIMING MATERIALS:

Adolph Mehlich, North Carolina State College, Raleigh, N. C., has been appointed Associate Referee on Exchangeable Potassium in Soils, in place of J. Fielding Reed.

COSMETICS:

Henry Kramer, Food and Drug Administration, Baltimore 2, Md., has been Appointed Associate Referee on Deodorants and Anti-perspirants, in place of S. H. Newburger.

George McClellan, Food and Drug Administration, New Orleans 16, La., has been appointed Associate Referee on Depilatories, in place of S. H. Newburger.

SPICES AND OTHER CONDIMENTS:

A. N. Prater, Gentry Inc., Los Angeles 54, Calif., has been appointed Associate Referee on Seeds and Stems in Ground Chili Peppers.

Samuel Alfend, Referee, has been appointed as Chief of the Kansas City District, with address at Kansas City, Mo.

CORRECTIONS IN FEBRUARY JOURNAL**Report on Changes in Methods, Vol. 32, No. 1**

Page 89. The method for "Lead in Lakes (Aluminum) of Coal-Tar Colors" should include paragraph under "Apparatus" given in *This Journal*, 31, 678 (1948).

Pages 94, 95. Fruit and Fruit Products, item (3) and (4) should read: "The following method was adopted as tentative," instead of "official, first action."

Page 95, last line of method for Water-insoluble Solids (rapid method), change "+" to "÷".

Page 104. Preparation of Assay Solution, line 8, change "30 min." to "15 min."; line 15, change "to" to "of."

Page 109, footnote (6), insert "not" before "necessary."

CONTRIBUTED PAPERS

LIMITATIONS OF THE "MODIFIED KJELDAHL METHOD" FOR DETERMINING THE NITRATE NITROGEN IN NITRATE-CHLORIDE MIXTURES*

By H. K. WHITE and O. W. FORD, *Associate Referee* (Purdue University
Agricultural Experiment Station, West Lafayette, Indiana)†

The official method for determining the nitrate nitrogen in fertilizers (1) has been criticized because of reported losses of nitrogen in this procedure when chlorides are present. It has been reported that these take place through a series of reactions ending with the formation of nitrosyl chloride (2, 3, 4).

Some investigators have developed new and modified procedures because of this failure of the Kjeldahl method (2, 3).

A review of the literature does not reveal the limits of the official method, or what ratio of nitrate to chloride it can satisfactorily determine. In the first phase of our work this ratio has been determined. Since significant losses do occur as reported (2, 3, 4), further work was undertaken to establish where these losses take place. Because chlorine is evolved from some mixtures, indicating the presence of a redox reaction which could possibly cause losses of nitrogen, some work was undertaken to determine the existence and extent of these losses.

EXPERIMENTAL

To establish the limits of the official method (1) for nitrate-chloride mixtures, a series of determinations were made on mixtures of analytical grade sodium nitrate and potassium chloride in decreasing ratios, by the official method (2.27 part 2) as revised by Ford (5). This procedure, used for the routine determination of total nitrogen in fertilizers in our control laboratory, is as follows:

(1) Prepare, at least $\frac{1}{2}$ day in advance, the special sulfuric acid reagent: a solution of salicylic acid in concentrated sulfuric acid, containing 2 g per 30 ml. (Low results are obtained if solution is not complete.)

(2) Place 0.7 to 3.5 g of the sample in a long neck Kjeldahl flask and add 40 ml of special sulfuric acid. Let stand at least one-half hour; or, in case of nitrate salts, until the salts are dissolved. (When analyzing fertilizer mixtures containing nitrate salts and limestone, keep mixture cool until the action has ceased and solution is complete. Otherwise loss of oxides of nitrogen may result.)

(3) Gradually add 2 g of zinc dust, shaking flask at the same time.

* Purdue University Agricultural Experiment Station Journal Paper No. 377.

† Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11-13, 1948.

(In the case of nitrate salts, mixture should be kept cool by holding flask under cold water tap until action is complete.)

(4) Heat the flask over low flame or on an electric rack for 5–10 minutes. Turn off heater, add 0.7 g HgO and 10 g K₂SO₄, and reheat until liquid in the flask is colorless or nearly colorless (1½–2 hours, in the case of electric digestion).

(5) Cool and, just before caking, add ca 200 ml of water. Precipitate mercury with 25 ml of 4% K₂S soln.

(6) After cooling, distill as in 2.24, using 100 ml of 40–45% NaOH.

(7) Conduct blank test on the reagents, following same procedure as outlined above.

TABLE 1.—*Total nitrogen found in nitrate-chloride mixtures*

RATIO NaNO ₃ :KCl	FORD'S METHOD	DEVARDA METHOD	RATIO NaNO ₃ :KCl	FORD'S METHOD	DEVARDA METHOD
1:0	16.41	16.42	5:2	16.37	16.43
	16.34	16.42		16.37	16.47
	16.34	16.42		16.32	16.42
	16.38			16.25	
	16.38				
	16.35				
	16.37				
Average	16.37	16.42		16.33	16.44
10:1	16.38		2:1	16.24	16.49
	16.43			16.24	16.49
	16.24			16.14	16.41
	16.43			16.02	
Average	16.37			16.16	16.46
5:1	16.37		1:1	16.13	16.47
	16.38			16.13	16.41
	16.25			16.09	16.46
	16.27			16.07	
				16.06	
Average	16.32			16.10	16.45
10:3	16.35	16.41	1:2	15.39	16.47
	16.24	16.42		15.44	
	16.39			15.43	
				15.41	
				15.43	
				15.40	
Average	16.33	16.42		15.42	16.47

Compounds of the types formed as intermediates in the official procedure were analyzed, with and without chloride added, to establish where the losses of nitrogen in the presence of chlorides occur. No significant differences were found (Table 2). The results of the work on the various mixtures are shown in Table 3.

DISCUSSION OF RESULTS

As shown in Table 1 the losses of nitrogen increased as the concentration of nitrate with respect to chloride decreased. However in ratios in excess of 5 to 2, the loss of nitrogen was less than 1 per cent.

The work with the intermediate type compounds definitely establishes

TABLE 2.—*Total nitrogen found in intermediate type compounds with and without potassium chloride*

SAMPLE	WITHOUT ADDED CHLORIDE	WITH ADDED CHLORIDE RATIO (SAMPLE: KCl) 1:2
NH ₄ Cl (Analytical Grade)	26.12	26.15
	26.15	26.18
	26.11	26.15
p-aminobenzoic acid (MP 187–188)	10.21	10.21
	10.21	10.21
	10.25	10.25
		10.21
5-nitrosalicylic acid (MP 228°C.)	7.48	7.41
	7.49	7.47
	7.45	7.47
	7.47	

that these losses occur in the nitration step. The ability to obtain consistent results, both with and without chloride, with (1) an ammonium salt, (2) an aromatic amine, and (3) an aromatic nitro compound, shows that these losses take place before digestion, before reduction, and before nitration, respectively. Good recovery with the 5-nitrosalicylic acid indicates that the loss occurs before the nitration is completed, and establishes the fact that, once the salicylic acid is nitrated, the subsequent reduction can be performed without loss of nitrogen.

A chloride would not be expected to interfere with this step unless it were oxidized to chlorine. Chlorine could compete with the nitrate ions in the nitration reaction, with the formation of chlorosalicylic acid. However, in the presence of an excess of salicylic acid, the losses from this competitive reaction are insignificant compared to those incurred in the oxidation of chloride to chlorine. The redox reaction between the chloride and nitrate ions is evidently developed when the salicylic-sulphuric

TABLE 3.—*Total nitrogen found in high nitrate-chloride complete fertilizer mixtures*

	PER CENT	PROCEDURE			
		DATE OF DETERMINATION			
		FORD'S METHOD			DEYARDA
		5/11/48	5/23/48	9/9/48	9/10/48
<i>MF 530 (Prepared 5/11/48)</i> <i>Composition</i>					
NH ₄ NO ₃	33.3	10.92	10.99	10.79	10.95
KCl	33.3	10.99	11.06	11.00	11.14
Treble superphosphate	33.3		10.06	11.89	11.03
				11.09	
				11.10	
Average		10.96	11.04	10.98	11.04
<i>MF 531* (Prepared 5/11/48)</i> <i>Composition</i>					
NH ₄ NO ₃	25	8.47	8.75	8.83	8.88
KCl	25	8.33	8.75	8.81	8.92
CaCO ₃ (ppt).	25		8.68	8.81	9.04
Treble superphosphate	25			8.82	
				8.79	
Average		8.40	8.73	8.81	8.95
<i>MF 532 (Prepared 5/11/48)</i> <i>Composition</i>					
NH ₄ NO ₃	33.3	10.78	10.78	10.67	10.86
KCl	33.3	11.16	11.06	10.64	10.90
20% superphosphate	33.3		10.85	10.79	10.86
				10.99	
				11.14	
Average		10.97	10.90	10.85	10.87
<i>MF 533 (Prepared 5/23/48)</i> <i>Composition</i>					
NaNO ₃	33.3		5.32	5.21	5.57
KCl	33.3		5.32	5.24	5.56
20% superphosphate	33.3		5.32	5.36	
				5.28	
				5.22	
				5.34	
Average			5.32	5.35	5.56

* No chlorine detected.

acid mixture is added, and results in the evolution of various oxidation and reduction products. This would seem to indicate that, in fertilizer containing nitrates, any substance which can be oxidized by the nitrate ion is a potential source of nitrogen losses when nitrogen is determined by the modified method.

The results of the study of mixtures indicate that no appreciable losses of nitrogen occur during storage for extended periods (see Table 3). In the sample containing calcium carbonate as well as treble superphosphate (MF 531), an apparent gain in nitrogen occurred which was probably due to the evolution of carbon dioxide from the mixture. However, in the other samples losses are shown, when the modified and Devarda procedures are compared, which are of the order expected in the presence of chloride and nitrate ions.

SUMMARY

If the ratio of nitrate (NaNO_3) to chloride (KCl) is less than 5 to 2, an appreciable loss of nitrogen may be expected when analysis is made by the official Kjeldahl method as modified to apply to nitrates. This loss occurs during nitration, and is believed to be due to the oxidation of the chloride by the nitrate ion. These ions are formed in the first step of the procedure, the addition of the sulphuric-salicylic acid mixture, and react to form volatile oxides which escape or cannot be reduced by the official method. No appreciable losses take place in nitrate-chloride mixtures when these are stored for an extended period.

LITERATURE CITED

- (1) *Official and Tentative Methods of Analysis*, 2.27, part 2, p. 27, 1945.
- (2) DYER and HAMENCE, *Analyst*, **63**, 866 (1938).
- (3) SHUEY, P. McG., *Ind. Eng. Chem., Anal. Ed.*, **19**, 882-883 (1947).
- (4) PRINCE, A. L., *This Journal*, **30**, 235 (1947).
- (5) ETHEREDGE, M. P., Associate Referee for Nitrogen, Instructions to Collaborators, March, 1948. (page 241, this issue).
- (6) *Official and Tentative Methods of Analysis*, 2.31, p. 28, 1945.

SEMI-MICRO PHENOL COEFFICIENT METHODS FOR TESTING QUATERNARY AMMONIUM DISINFECTANTS*

By GEORGE S. WARNER and MICHAEL J. PELCZAR, JR. (Department of Bacteriology, University of Maryland, College Park, Md.) and L. S. STUART (Production and Marketing Administration, U. S. Department of Agriculture, Washington, D. C.)

The subject of the appraisal of bactericidal efficiency of quaternary ammonium compounds has been excellently reviewed by Rahn and Van

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11-13, 1948.

Eseltine (1). Among other tests, modifications of the official A.O.A.C. phenol coefficient method have been proposed to eliminate the random sampling error of the 4 mm transfer loop used in that method, and thus furnish a more accurate index to the actual killing concentration of such disinfectants. Of these, the method recently proposed by Klarmann and Wright (2) for the evaluation of quaternary ammonium germicides has received considerable attention. This method employed 1/10 of the quantities of medicant and culture used in the A.O.A.C. method; and sub-culture was made of the entire volume of disinfectant-culture mixture at the conclusion of the exposure time interval. It is obviously a more critical test than the A.O.A.C. phenol coefficient test insofar as the determination of the actual killing concentration is concerned, since the sub-culture sample is some 25 times larger and the ratio of apparatus surface to medicant volume is appreciably higher. In general, therefore, lower results might be expected depending among other things on the degree of surface activity of the disinfectant being tested. For this reason results secured with methods of this type are not strictly comparable with results secured by the official A.O.A.C. method.

Such methods may, however, prove to be especially useful in determining actual end points for complete kill with disinfectants of the type that kill a very high proportion of the test culture cells within a very short exposure interval, but which kill the few remaining viable cells at a progressively slower rate. With disinfectants of this type, the random sampling error with the 4 mm loop sub-sampling procedure is great enough at the longer exposure intervals to make it exceedingly difficult to determine exact concentration, death time, and end points with any degree of accuracy. The behavior of quaternary ammonium germicides in this respect has led to a great deal of criticism of the official A.O.A.C. method, and to considerable conjecture relative to their true killing or disinfectant concentrations.

A representative number of these compounds were studied, therefore, by both the official phenol coefficient procedure, employing trypticase broth and a modified semi-micro method embodying the general principles as set forth by Klarmann and Wright (2) to establish the relationship of results secured by the two methods and to establish more definitely the actual 10 minute killing concentration of such compounds.

EXPERIMENTAL

TEST ORGANISMS

Eberthella typhosa Hopkins and *Staphylococcus aureus* 209 were employed as the test organisms. Since the above names have been in common usage, this older nomenclature has been retained in preference to that of *Salmonella typhosa* and *Micrococcus pyogenes* var. *aureus* proposed in the Sixth Edition of Bergey's Manual of Determinative Bacteriology (3).

MEDIA AND METHODS

The official A.O.A.C. phenol coefficient procedure was employed exactly as outlined in the official methods except that trypticase broth was substituted for F.D.A. broth. The composition of the trypticase broth was:

Trypticase.....	10 g
Beef extract.....	5 g
NaCl.....	5 g
Distilled water.....	1000 ml

In the semi-micro method, trypticase broth was also used for propagating the test cultures and a trypticase-Tween 80-Azolectin broth was used for subculturing. The latter was designed and employed in sufficient volume to neutralize both the bactericidal and bacteriostatic effects of the entire quantity of the quaternary ammonium germicide used for each exposure period. The lecithin acted as a neutralizing agent or antidote, and the Tween 80 as a dispersing agent. The formula for this medium was:

Trypticase.....	10 g
Beef extract.....	5 g
NaCl.....	5 g
Azolectin.....	1.5 g
Tween 80.....	10 g
Distilled water.....	1000 ml

Preparation of the medium was accomplished in the following manner: The Azolectin and Tween 80 were added to 400 ml of hot distilled water, and the mixture was boiled 10 minutes to effect a clear solution. The remaining ingredients were dissolved by boiling in 600 ml of distilled water and were added to the clear Azolectin-Tween 80 dispersion. The final solution was boiled for 10 minutes, made to a volume of 1000 ml with distilled water, and adjusted to pH 7.1, tubed in 10 ml amounts and autoclaved for 20 minutes at 15 lbs (121°C).

This medium is essentially that initially employed by Quisno, Gibby, and Foter (4). It contains, however, a greater concentration of lecithin and Tween 80. This was found to be necessary to completely neutralize the large quantity of quaternary ammonium germicide present in the sub-culture sample employed. The adequacy of the exact level was determined in a series of preliminary tests. An example of the type of results secured in these tests is given in Table 1.

In this particular study, alkyl dimethyl benzyl ammonium chloride¹ was added to culture tubes containing 10 ml of sterile trypticase-Tween 80-Azolectin broth to give the dilutions indicated (Table 1). The tubes were then conditioned in a 20°C. water bath for 5 minutes and inoculated with 0.1 ml of a 24 hour broth culture of *S. aureus* 209 held for 10 minutes at this temperature and then incubated at 37.5°C. for 48 hours. Condition-

¹ The alkyl radicals in this compound range from C₈H₁₇—C₁₈H₃₅.

ing and holding of these tubes at 20°C. for 10 minutes seemed necessary, since this one study was used to check on both bactericidal and bacteriostatic effects. Growth in the tubes, as evidenced by turbidity, was recorded at 24 and 48 hour intervals. At the 48 hour interval, 0.1 and 1.0 ml sub-samples were plated in trypticase agar and these plates incubated at 37°C. for 48 hours to confirm readings based on turbidity evaluations.

Inasmuch as *S. aureus* 209 is an organism especially susceptible to the bactericidal and bacteriostatic effects of alkyl dimethyl benzyl ammonium chloride,¹ such results indicated that the trypticase-Tween-80-Azolectin sub-culture broth would act as an effective antidote for quantities of quaternary ammonium germicides up to .000167 gm where .015 gm of Azolectin and 0.1 gm of Tween 80 were present. It should be noted,

TABLE 1.—*Effect of 1 percent Tween 80 and 0.15 per cent Azolectin on growth of Staphylococcus aureus 209*

DILUTION OF ALKYL DIMETHYL BENZYL AMMONIUM CHLORIDE IN SUB-CULTURE MEDIUM	TRYPTICASE TWEEN 80 AZOLECTIN BROTH GROWTH AT 37.5°C. AS EVIDENCED BY TURBIDITY IN SUB-CULTURE BROTH		AGAR PLATE COUNTS OF 48 HOURS BROTH CULTURES	
	24 HRS	48 HRS	1.0 ML	0.1 ML
1:3000	—	—	0	0
1:5500	—	—	0	0
1:6000	—	—	0	0
1:6500	—	+	TNTC*	TNTC
1:7000	+	+	TNTC	TNTC
1:7500	+	+	TNTC	TNTC
1:10,000	+	+	TNTC	TNTC

* TNTC = Colonies too numerous to count.

however, that the neutralizing effect of this medium appeared to be completely lost when the quaternary salt concentration passed a certain level, and that this level was so sharply defined as to indicate direct quantitative relationships between the neutralizing agent present and the quaternary ammonium salt.

The technique of the semi-micro method employed in these studies varied from that used by Klarmann and Wright (2) in the amount of medicant mixture, in the amount and composition of the sub-culture medium, and in the method of preparation of the medicant mixture. The details are as follows: In preparation for the actual test, an excess quantity (5 ml) of each dilution of medicant, selected to cover the killing range, was pipetted into separate sterile (20×150 ml) test tubes and tempered for 5 minutes in the 20°C. water bath. For the actual 5 minute test interval, 0.1 ml of a 22 to 26 hour broth culture of the test organism, previously shaken for fifteen minutes to break up clumps, was added to each of a series of 10 sterile test tubes labelled to indicate the selected dilution of

medicant contained. This series of medicant tubes was placed in the water bath parallel to the tubes containing the corresponding dilutions of medicant. One ml was pipetted from each medicant dilution into the corresponding medicant tube containing the test culture aliquot, a 30 second interval being employed between each dilution transfer. Five minutes after the dilution of medicant was added to the medicant tube, 10 ml of sterile trypticase-Tween 80-Azolectin broth was added aseptically, the tube shaken briefly and placed in a suitable rack. As soon as this process was completed with the entire dilution series, all tubes were vigorously shaken and placed in the incubator at 37°C. for 48 hours. The foregoing procedure was repeated in its entirety for both the 10 and 15 minute exposure intervals.

For comparative purposes the A.O.A.C. technique using trypticase broth was carried out concurrently each day that the semi-micro studies were made.

In both the semi-micro method and the A.O.A.C. method with trypticase broth, the maximum lethal concentration or the minimum lethal dilution of the test compound or of phenol was established as that dilution which killed the test organism in 10 minutes, but not in 5 minutes.

RESULTS

Phenol coefficient values have been obtained for four quaternary ammonium compounds by the A.O.A.C. method using trypticase broth and a semi-micro method described.

In Tables 2 and 3, the minimum lethal concentrations of the four quaternary ammonium germicides studied for *E. typhosa* and *S. aureus* 209 determined by the A.O.A.C. method with trypticase broth, and by the semi-micro method, are compared. Phenol coefficient values have been calculated from results obtained with both techniques.

It can be seen that the minimum lethal concentration found in the semi-micro procedure was considerably lower, with both test organisms, for all four quaternary ammonium germicides than that found by the A.O.A.C. method using trypticase broth. The minimum lethal concentration with phenol was the same by both the semi-micro and the modified A.O.A.C. method in the case of both test organisms. Obviously, therefore, the phenol coefficient values secured by the semi-micro method are considerably lower than those secured by the A.O.A.C. technique.

If the phenol coefficient values secured by the A.O.A.C. technique for the quaternary ammonium germicides against *E. typhosa* are multiplied by 20, as is the presently accepted standard procedure in determining the safe use dilution for disinfectants, it can be seen that the safe use dilution indicated is higher in 2 instances and lower in the other 2 instances than the minimum 10 minute lethal concentration determined by the semi-micro method. The average safe use dilution, indicated by phenol coeffi-

TABLE 2.—*Phenol coefficient values of four quaternary ammonium compounds determined by the A.O.A.C. method using Trypticase broth and a modified semi-micro technique using Eberthella typhosa Hopkins at 20°C.*

DISINFECTANT	A.O.A.C. TECHNIQUE WITH TRYPTICASE BROTH		MODIFIED SEMI-MICRO TECHNIQUE	
	MINIMUM CON- CENTRATION LETHAL IN 10 MIN.	PHENOL COEFFICIENT	MINIMUM CON- CENTRATION LETHAL IN 10 MIN.	PHENOL COEFFICIENT
Phenol	1:90		1:90	
Alkyl-dimethyl benzyl ammonium chloride	1:15,000	166.6	1:2,000	22.2
Cetyl trimethyl ammonium bromide	1:15,000	166.6	1:2,300	25.6
p-di iso butyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride	1:10,000	110.8	1:2,800	30.8
Cetyl pyridinium chloride	1:6,500	72.2	1:1,500	16.6

TABLE 3.—*Phenol coefficient values of four quaternary ammonium compounds determined by the A.O.A.C. method using Trypticase broth and a modified semi-micro technique using Staphylococcus aureus 209 at 20°C.*

DISINFECTANT	A.O.A.C. TECHNIQUE WITH TRYPTICASE BROTH		MODIFIED SEMI-MICRO TECHNIQUE	
	MINIMUM CON- CENTRATION LETHAL IN 10 MIN.	PHENOL COEFFICIENT	MINIMUM CON- CENTRATION LETHAL IN 10 MIN.	PHENOL COEFFICIENT
Phenol	1:70		1:70	
Alkyl-dimethyl benzyl ammonium chloride	1:17,500	250	1:3,500	50
Cetyl trimethyl ammonium bromide	1:20,000	285.7	1:3,500	50
p-di iso butyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride	1:22,000	314.4	1:2,800	40
Cetyl pyridinium chloride	1:20,000	285.7	1:3,500	50

cient values by the A.O.A.C. method (except that trypticase broth was used), was 1:2581, whereas the critical killing dilution indicated by the semi-micro technique was 1:2150. It would seem, therefore, that the semi-micro method yields results more comparable with those secured by "Use dilution methods" than does the A.O.A.C. phenol coefficient method. For the present, at least, this seems to be the only practical interpretation which can be made; for there is not sufficient evidence available to intelligently use the lesser phenol coefficient values secured by this method in the calculation of safe use concentrations. Data must be compiled from practical use studies, comparing effects with 5% solutions of phenol and varying concentrations of quaternary ammonium germicides, to determine the proper conversion factor to employ before this would be feasible.

It is of interest to note that the phenol coefficient values secured by the A.O.A.C. method employed were considerably lower than those usually claimed or reported on the basis of tests by the official A.O.A.C. method when the original F.D.A. broth was used. This is attributed to the use of trypticase broth in the propagation of the test cultures employed, and tends to confirm the early observation of Brewer (5) that differences in the peptones employed in the culture media will cause significant differences in results secured with quaternary ammonium germicides by the A.O.A.C. phenol coefficient procedure. On the other hand, the trypticase broth used for sub-culturing did not contain Tween 80 and lecithin to neutralize the bacteriostatic effects of the quaternary ammonium germicides, and there is considerable evidence to indicate that if this had been done the results, with *S. aureus* 209 at least, would have been even lower. It is also possible that those secured with *E. typhosa* might have been low enough so that the phenol coefficient values secured by the A.O.A.C. technique would have indicated safe use dilutions considerably less in all instances than the critical killing dilutions found in the semi-micro method.

Both "time skips" and "dilution skips" were occasionally encountered in the semi-micro method tests. They were, however, much less frequently encountered than in tests using the A.O.A.C. technique. Thus, characteristic time-concentration killing end-points were somewhat easier to establish and duplicate. In this connection, it is especially interesting to report that the work sheets for the semi-micro tests generally indicated lower killing concentrations for the 15 min. exposure interval than for the 10 min. exposure interval. This seems to indicate that the small percentage of cells in test cultures, which survive the initial exposure to a given concentration of quaternary ammonium germicides are eventually killed on prolonged contact.

SUMMARY AND CONCLUSIONS

A semi-micro phenol coefficient method for determining the germicidal potency of quaternary ammonium disinfectants has been described. The

maximum lethal killing dilutions found by this method were not appreciably different from the safe use dilutions indicated by the A.O.A.C. phenol coefficient method, using trypticase broth, when the standard formula of 20 times the phenol coefficient found with *E. typhosa* was employed to determine the number of parts of water to add to one part of disinfectant for preparing a safe use dilution. These results suggest that the conditions of the semi-micro test described may have been severe enough to warrant its classification as a "use dilution test," but additional correlations with actual use studies will be necessary to determine whether this is true. If this is the case, then the results secured would indicate that the basic procedure of the official A.O.A.C. test may provide a reasonably reliable presumptive index to the germicidal potency of quaternary ammonium germicides if the sub-culture broth employed contains a neutralizer for bacteriostatic quantities of these germicides which may be carried over.

Critical quaternary ammonium germicide concentration killing times could be more easily established with the semi-micro method described than with the A.O.A.C. technique. For this reason, it may have practical value as a test method for confirming results secured by the A.O.A.C. phenol coefficient procedure or for initial assays when a fixed relation between the critical killing concentrations, or phenol coefficient values found therewith, and safe use dilutions can be established.

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VARIATIONS IN RESISTANCE IN *E. TYPHOSA* AND *S. AUREUS* WHEN MAINTAINED AS SPECIFIED ACCORDING TO THE OFFICIAL A.O.A.C. PHENOL COEFFICIENT METHOD*

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INTRODUCTION

The ability to maintain and propagate selected test cultures at standard and uniform resistance levels to phenol, is a fundamental necessity for the

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correct evaluation of chemical germicides by the official A.O.A.C. phenol coefficient method.

This method prescribes the maintenance of 30-day stock cultures of F.D.A. agar slants; and the preparation therefrom of 24-hour broth test cultures by employing 3 successive 24-hour serial transfers using F.D.A. broth.

It occasionally happens that test cultures prepared in this manner are found to possess levels of resistance outside the limits prescribed by the official test. This results not only in invalidating the results secured in the particular tests in question, but also disrupts the entire testing program until a procedure is worked out to re-establish the test cultures at the resistance levels desired.

The proper culture method to employ to re-establish a given resistance level in a test culture, is a moot question. It is well known that changes in culture methods, involving such environmental conditions as temperature, pH, and culture composition, may possibly result in permitting the establishment of permanent variant cells; so that sub-cultures occasionally possess characteristics varying widely from those of the mother culture. Therefore, it would seem essential to avoid procedures that employ such variables. If this is done, and the method employed is restricted to use of the specific media and temperatures stipulated in the official test, the only variations which could be encountered would be those, normal to the culture under the condition specified, that result from increases in population density or age.

Inasmuch as no actual data are available on the degree or permanency of variation in the specified cultures; namely, *Eberthella typhosa* (Hopkins Strain) and *Staphylococcus aureus* 209, resulting solely from culture age, this study was conducted to ascertain:

- (1) The normal variations in the resistance to phenol of F.D.A. broth test cultures prepared from 30-day agar stock cultures of the two test organisms as prescribed by the A.O.A.C. method.
- (2) The degree of permanence of the variation encountered in these cultures.
- (3) If methods, based on the investigation of culture variants resulting solely from culture age, are adequate for re-standardizing resistance to the desired level.

EXPERIMENTAL

TEST ORGANISMS

Eberthella typhosa (Hopkins) and *Staphylococcus aureus* 209 were employed as the test organism. As the above names are employed in the present official A.O.A.C. Method, this older nomenclature has been retained in preference to *Salmonella typhosa* and *Micrococcus pyogenes* var. *aureus* proposed in the Sixth Edition of Bergey's Manual of Determinative Bacteriology.

MEDIA, METHODS, AND RESULTS

The only media employed were F.D.A. Agar and F.D.A. nutrient broth prepared exactly as stipulated in the A.O.A.C. method.

Individual 30-day agar slant stock culture of *E. typhosa* and *S. aureus* were washed off and suspended in sterile physiological saline solution. Serial dilution plates were made from these suspensions in F.D.A. Agar and incubated for 48 hours at 37°C. Plates containing between 30 and 100 discrete colonies were then selected from each series of dilutions, and all others discarded. From these plates 30 typical colonies were picked at random and transferred to individual tubes of sterile F.D.A. broth. These tubes were incubated at 37°C. for 24 hours, after which transfers were made to fresh tubes of broth incubated in the same manner, and thence into a third set of broth tubes incubated at 37°C. for 24 hours. The 24-hour cultures resulting from this third set of transfers were then examined for their resistance to dilutions of phenol by the procedure outlined in the official A.O.A.C. method. The highest dilution which killed the test organism within 10 minutes, but not in 5, at 20°C. was considered to be the critical killing dilution.

This procedure was employed with three consecutive 30-day agar slant stock cultures of both test organisms, providing in each case 90 selected and experimentally standardized sub-cultures for examination relative to phenol resistance. It is believed that these sub-cultures provided a representative sampling of the stock cultures maintained according to the specifications given in the official A.O.A.C. method. The results are shown in Table 1.

TABLE 1.—Critical killing dilutions of phenol for standardized 24-hour broth cultures of *E. typhosa* and *S. aureus*

PHENOL DILUTION USED	NUMBER OF CULTURES OF <i>E. typhosa</i> KILLED IN 10 MIN. BUT NOT IN 5 MIN.				NUMBER OF CULTURES OF <i>S. aureus</i> KILLED IN 10 MIN. BUT NOT IN 5 MIN.			
	SERIES			TOTAL	SERIES			TOTAL
	1	2	3		1	2	3	
1-40	0	0	0	0	0	0	0	0
1-45	0	0	0	0	0	0	0	0
1-50	0	0	0	0	1	0	0	1
1-55	0	0	0	0	0	3	0	3
1-60	0	0	0	0	8	18	9	35
1-65	1	1	0	2	7	9	14	30
1-70	1	1	0	2	13	0	7	20
1-75	0	1	0	1	1	0	0	1
1-80	17	4	0	21	0	0	0	0
1-85	3	15	1	19	0	0	0	0
1-90	8	8	15	31	0	0	0	0
1-95	0	0	8	8	0	0	0	0
1-100	0	0	4	4	0	0	0	0
1-105	0	0	1	1	0	0	0	0
1-110	0	0	1	1	0	0	0	0
Total No. of Cultures	30	30	30	90	30	30	30	90

The results in Table 1 indicate that fresh 24-hour broth test cultures of *E. typhosa* prepared as prescribed by the A.O.A.C. method may possess resistances to phenol dilutions varying from 1-65 to 1-110, and that similar test cultures of *S. aureus* may show resistant levels varying from dilutions of 1-50 to 1-75. The frequency of the occurrence of cultures

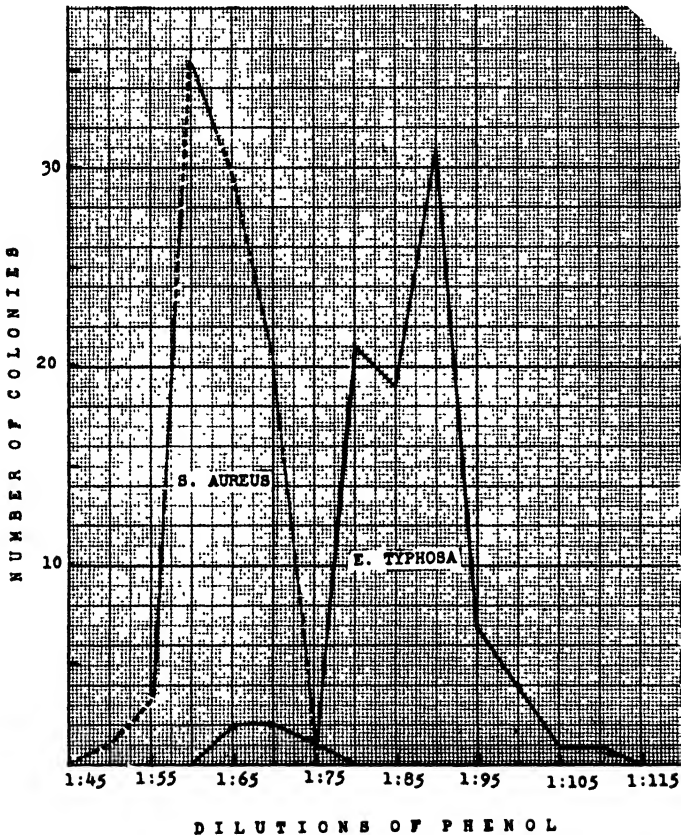


FIG. 1.—Frequency distribution curves on the critical killing dilutions of phenol for *E. typhosa* and *S. aureus*.

outside the prescribed resistance levels of 1-60 to 1-65 for *S. aureus* is small. With *E. typhosa*, however, quite a large number of cultures were obtained with resistances somewhat greater than the greatest concentration or lowest dilution specified in the test, and the range in dilution resistance was considerably broader than with *S. aureus*. These results seem to indicate that cultures of this organism contain cells which vary widely in resistance, and have a tendency to possess a slightly greater resistance in freshly prepared broth test cultures than is specified in the official test. In Figure 1, the data in Table 1 are presented in the form of distribution curves for both organisms.

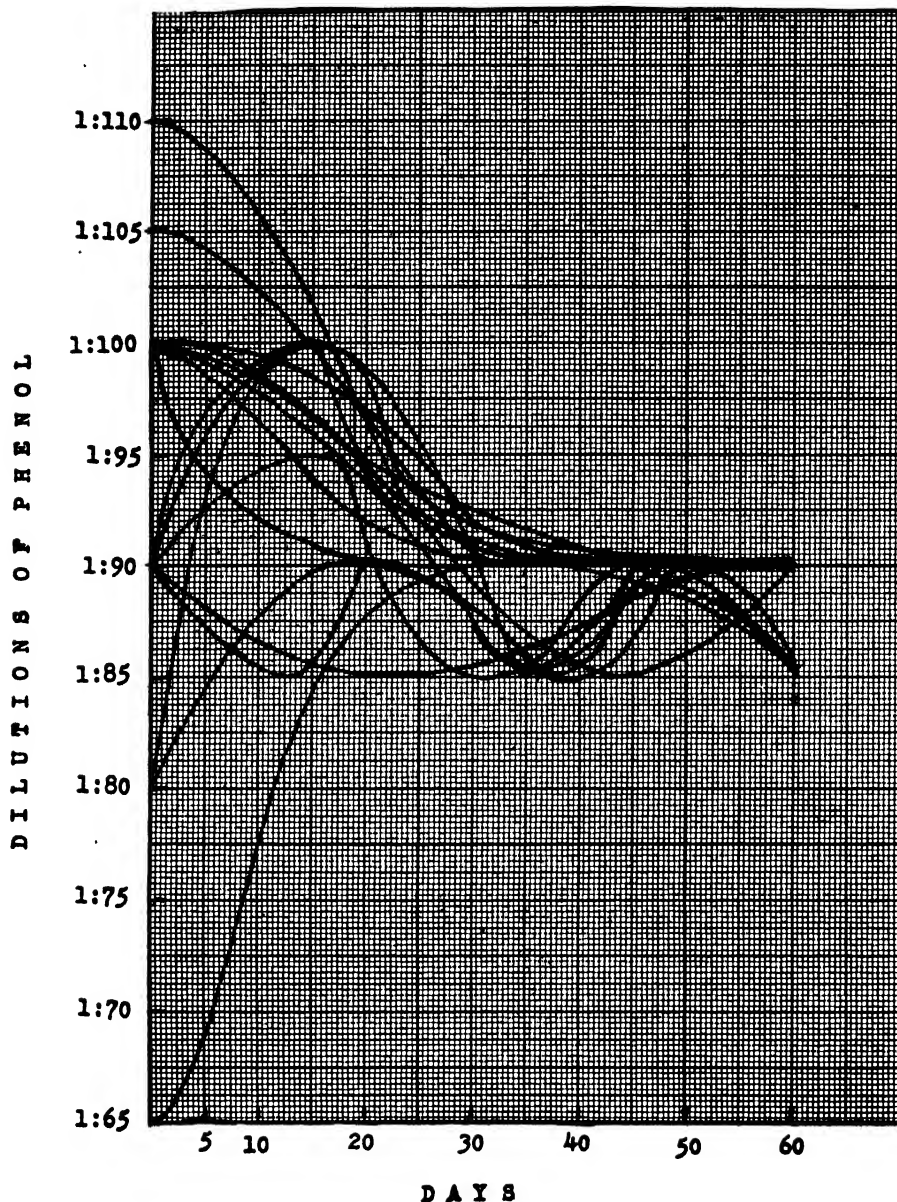


FIG. 2.—Changes in resistance levels at 20°C. of experimentally standardized test cultures of *E. typhosa* in broth maintained with 24-hour serial transfers.

All 24-hour test cultures were maintained in broth at 37°C., and daily transfers were made until the initial resistance level had been ascertained. Broth cultures representative of each resistance level recorded were then selected for further study, to determine the influence of continued propagation in F.D.A. broth at 37°C. (with 24-hour transfers) on the constancy

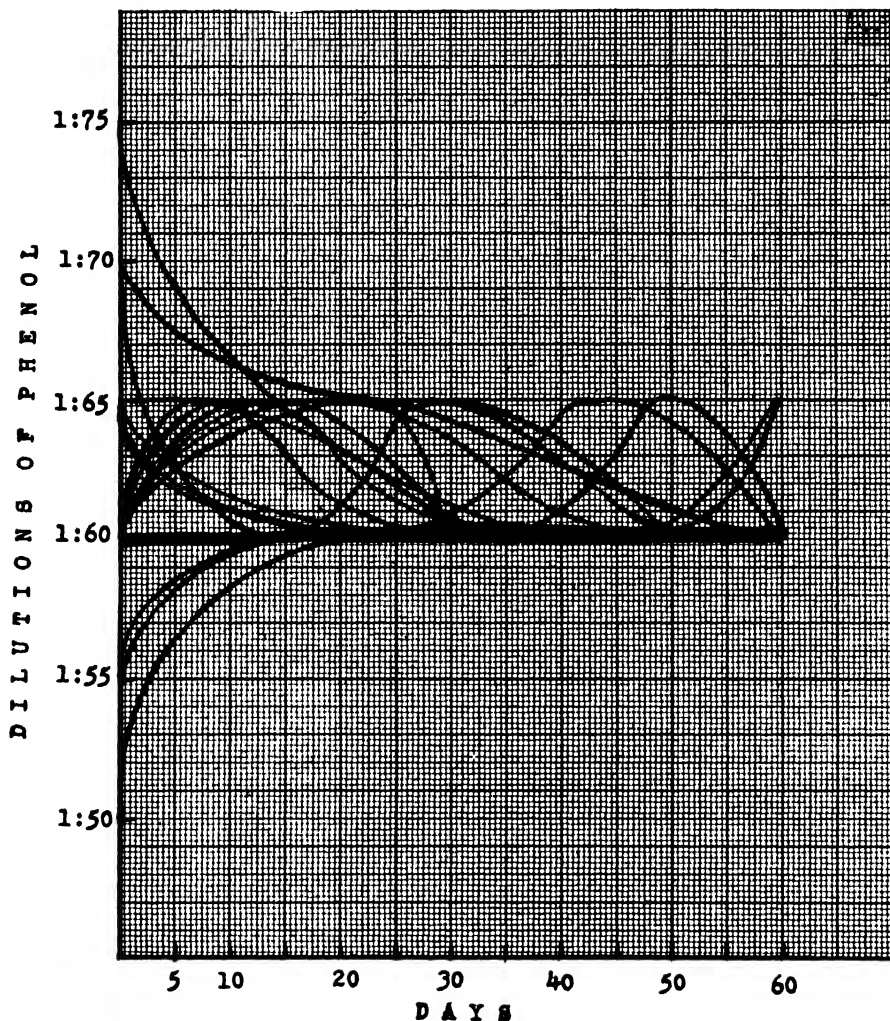


FIG. 3. - Changes in resistance levels at 20°C. of experimentally standardized test cultures of *S. aureus* in broth maintained with 24-hour serial dilutions.

of the initial resistance levels recorded. All other experimental test cultures were discarded.

Phenol resistance levels were determined at various intervals on each test culture so maintained for a period of 60 days. The results of these studies with *E. typhosa* are summarized in graphic form in Figure 2 and those for *S. aureus* similarly presented in Figure 3.

From study of the 16 curves in Figure 2, representing the resistance levels found with 16 individual selected cultures of *E. typhosa* of varying initial resistance levels maintained in F.D.A. broth at 37°C. in the 24-hour transfers over a 60-day period, it can be seen that all 16 cultures eventually

developed resistance levels to dilutions of from 1-85 to 1-95. These levels are within the range of resistance to phenol prescribed for this organism in the test. One culture with an initial resistance level of 1-65 decreased in resistance to 1-85 within 16 days. Two with an initial resistance of 1-80 decreased to the desired level within one week. On the other hand, cultures with low initial resistance levels of 1-100, 1-105 and 1-110, all increased in resistance to the 1-95 dilution level within 26 days. Some cultures, which on initial standardization possessed the 1-90 dilution resistance, temporarily lost this resistance but regained it eventually after continued 24-hour transfers. On the whole, then, it appears that these findings tend to substantiate the conclusion indicated in Table 1 and Figure 1: that cultures of this organism, when maintained as specified in the test, contain a predominantly large number of cells within the resistance range of 1-85 to 1-95.

Examination of the 21 curves representing the resistance levels of the selected strains of *S. aureus* (Figure 3) shows that all 21 cultures developed resistances to phenol dilutions of 1-60 or 1-65, as specified for this organism in the official test. One strain with an initial resistance level at a phenol dilution of 1-50 decreased in resistance in the 1-60 dilution level within 21 days. Two with an initial resistance level at 1-55 decreased to the 1-60 level within 16 days. Five with an initial resistance level at 1-60 remained at this level for the entire 60 days while 6 others vacillated between the levels of 1-60 to 1-65 over this period. None of the 4 cultures which possessed an initial resistance level at 1-65 remained constantly at this level for the entire 60 day period, but all 4 retained a resistance at either 1-60 or 1-65 over the entire period. The 2 cultures possessing an initial resistance level at 1-70 and the 1 culture with an initial resistance at 1-75, rapidly increased in resistance to levels at 1-65 and 1-60 when transfers at 24-hour intervals were continued. These findings confirm the results indicated in Table 1 and Figure 1, that the mean resistance level of cells of this organism to phenol is at the 1-60 dilution.

Results with 24-hour cultures of both organisms derived from cultures maintained in F.D.A. broth at 37 C. for 60 days, with transfers at 48 and 72-hour intervals, were also secured. The resistance levels to phenol with such cultures were not constant, and many possessed resistance levels outside the ranges prescribed in the official method.

DISCUSSION

From the results secured in this study, it appears that the procedure described for maintaining and propagating test cultures in the official A.O.A.C. method, will provide test cultures of the prescribed resistance in at least 64 out of 100 cases of *E. typhosa* and 70 out of 100 cases of *S. aureus*. It is quite clear, however, that some cultures may be obtained possessing an initial resistance in each instance outside of the resistance

ranges tolerated. On the other hand, if these cultures that do not comply in resistance to the standards stipulated in the methods are continually maintained, with 24-hour transfers, in F.D.A. broth at 37°C., they will eventually develop the resistance levels prescribed. On the whole then the official procedure for maintaining and propagating the test culture as now outlined seems to be both fundamentally sound and practical.

Although a large number of experimentally standardized subcultures were employed in these studies, it is probable that the number used actually was too small from a statistical standpoint to yield frequency distribution curves of absolute accuracy. However, the curves established do probably give a fairly representative picture of the general character of the resistance frequency distribution in each instance. They indicate that resistance with *S. aureus*, when propagated according to the standard procedure, is highly characteristic at dilutions of phenol of 1-60 and 1-65. *E. typhosa* does not appear to possess the sharply defined characteristic resistance level possessed by *S. aureus* 209, as quite large numbers of test cultures of *E. typhosa* were secured at resistance levels of the 1-80, 1-85 and 1-90 dilutions of phenol and some at such widely variable dilutions as 1-65 to 1-110, in the initial standardizations. This was apparently appreciated by the authors of the test, since the tolerance with *E. typhosa* is greater than with *S. aureus* 209.

The results of continued propagation, with 24-hour transfers in F.D.A. broth at 37°C., of selected experimentally standardized cultures over the entire resistance ranges observed, together with the initial standardizations for both cultures, tend to emphasize the fact that the resistances indicated as characteristic by the frequency distribution curves are characteristic and fixed properties of the 2 test cultures.

No permanent variation from these characteristic resistance levels was encountered in these studies with either culture. However, attention should be called to the fact that this does not eliminate the possibility that such permanent variation might occur if such variable environmental conditions as changes in pH, media composition and temperature, were employed. It should also be reported here that no R (rough) variants of *S. aureus* 209 were encountered in these studies, and that all experimentally standardized test cultures with this organism were propagated from S. or smooth type colonies.

Continuous propagation by the use of uninterrupted 24-hour transfers in F.D.A. broth at 37°C., would appear to be the most simple and convenient method for re-standardizing these 2 specified test cultures. If time does not permit this procedure to be followed, dilution plates by the use of F.D.A. agar may be made from the stock cultures and incubated for 48 hours at 37°C., from which 10 single colony isolates can be transferred to individual tubes of F.D.A. broth. If the resulting cultures are transferred serially at 24-hour intervals for 3 successive days, and phenol

resistance is then determined, a number of test cultures which possess the exact resistance desired will be obtained. It would appear that the latter procedure, from the standpoint of avoiding permanent variations from the mother culture, can be considered as a relatively safe one for the restandardization of test culture resistance with both *E. typhosa* and *S. aureus* 209. No permanent variants were encountered in these studies, in which a technique similar to that in the initial standardizations of experimental test cultures was employed.

Constancy of behavior is particularly important in the selection of a test culture. With respect to phenol resistance, the specific cultures of *E. typhosa* and *S. aureus* 209 used in the studies reported herein and selected for use in the official A.O.A.C. phenol coefficient method, appear to possess relatively constant characteristics. This undoubtedly accounts in part for the successful use of this test for such a long period of time.

Many other cultures have been, and are being, suggested for use as test cultures in the evaluation of chemical germicides. It would seem essential that each culture proposed for this use be studied carefully to determine procedures of maintenance and propagation which will prevent the development of permanent variants. The procedure outlined in the A.O.A.C. phenol coefficient method for maintaining and propagating *E. typhosa* (Hopkins strain) and *S. aureus* 209 appears to be adequate in this respect.

SUMMARY AND CONCLUSION

1. The procedure as now outlined in the official A.O.A.C. phenol coefficient method for maintaining and propagating test cultures of *E. typhosa* (Hopkins) and *S. aureus* 209 at standard resistance levels to phenol has been studied and found to be adequate in most respects.¹

2. The normal variations in cultures of *E. typhosa* (Hopkins) and *S. aureus* 209 when maintained and propagated according to the prescribed procedure have been recorded. With 90 freshly prepared 24-hour broth cultures from 30-day stock slant cultures employing 3 successive F.D.A. broth serial transfers at 37°C., the resistance to phenol dilutions varied with *S. aureus* 209 from 1-50 to 1-75, and with *E. typhosa* from 1-65 to 1-110. These resistance levels are somewhat wider than the 1-60 and 1-65 dilutions specified for *S. aureus* 209 and 1-85 to 1-95 dilutions specified for *E. typhosa* (Hopkins) in the test. However, with continued serial transfers at 24-hour intervals all experimental test cultures rapidly developed a resistance to phenol within these specified levels.

3. The phenol dilution resistance levels of 1-60 and 1-65 for *S. aureus* and 1-85 to 1-95 for *E. typhosa* specified in the test appear to be fairly constant and fixed characteristic properties of the two test cultures specified when maintained according to the A.O.A.C. procedures.

¹ *Official and Tentative Methods of Analysis*, Sixth Ed., 86-91, 1945.

4. Methods for restandardizing test cultures of *E. typhosa* (Hopkins) and *S. aureus* 209 to the phenol resistance levels desired have been described.

DETECTION OF TARTARIC ACID AND TARTRATES IN WINES

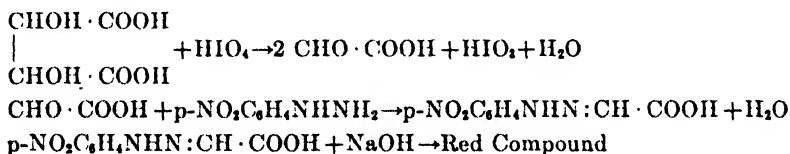
By ALEX P. MATHERS (Alcohol Tax Unit Laboratory, Bur. of Internal
Revenue, Washington 25, D. C.)

A review of the literature indicates some divergence of opinion as to the presence of tartaric acid and tartrates in fruits and berries other than grapes. Bigelow and Dunbar (1) state, "The burden of evidence in the literature seems to indicate that tartaric acid is not an ingredient of fruit juices except, of course, of grape juice." Winton (2) lists numerous references in discussing fruit acids, many of which are controversial with regard to the presence of tartaric acid in fruits other than grapes.

The procedure as outlined below is suitable for ordinary laboratory examination of wines, as ten per cent of grape wine in the presence of other fruit wines is easily detectable.

The selective oxidizing action of periodic acid on α , β -glycols to convert tartaric acid into glyoxylic acid is employed. Sprinson and Chargaff (3) in a study of periodic acid oxidations report the conversion of tartaric acid into glyoxylic acid, and the conditions for arresting the oxidation at this point.

The glyoxylic acid is converted into a p-nitrophenylhydrazone through the agency of p-nitrophenylhydrazine. The rearrangement of the p-nitrophenylhydrazone of glyoxylic acid in alkaline solution to give an intensely red-colored compound (4) (5), provides a delicate means of detecting small amounts of this compound.



The p-nitrophenylhydrazone of glyoxylic acid is easily obtained in crystalline form for microscopic identification, by evaporating the ethereal solution or alcoholic eluate containing the compound. The compound can be obtained in quite pure form by dissolving the crude residue in ether, extracting the ethereal solution with aqueous (2%) sodium bicarbonate solution, acidification of this aqueous solution with 5% sulfuric acid, and re-extraction with ether. The p-nitrophenylhydrazone of glyoxylic acid crystallizes in a fairly pure state upon evaporation of the ether.

REAGENTS

Activated Carbon (Darco S-51)

Neutral lead acetate (5% aqueous solution)

Neutral lead acetate (3% in glacial acetic acid)

Ammonium acetate (20% in glacial acetic acid)

Glacial acetic acid

Periodic acid (1% aqueous solution)

Sodium bisulphate (10% aqueous solution)

Sodium bisulphite (powdered)

Ethyl ether

Ethyl alcohol (anhydrous)

Sodium hydroxide (solid)

Powdered glass (Eimer and Amend) G-17

p-Nitrophenylhydrazine reagent: one gram of p-nitrophenyl hydrazine, or one of its salts, dissolved in 7.5 ml of concentrated sulfuric acid and diluted to 75 ml with ethyl alcohol (95%)

Asbestos fibers

Adsorption Tube:

Any small chromatographic column is satisfactory. Column is packed to height of 40 to 50 mm. with glass powder held in place by asbestos fibers. Tube may be made by sealing about 1" of 6 mm o.d. tubing to the bottom of a 16×150 mm test tube.

METHOD

Dilute 10 ml of wine to 50 ml with distilled water; add approximately one-half gram of activated carbon, warm on steam bath about ten minutes and filter into a centrifuge bottle. To the filtrate add 5 ml of 5% neutral lead acetate solution, centrifuge and decant the supernatant liquid. Wash the precipitate with 40 ml of distilled water again centrifuge and decant. Dissolve the precipitate in 5 ml of ammonium acetate solution, warming if necessary to effect solution. If a clear solution is not obtained due to coagulated material or inorganic salts, filter into a second centrifuge bottle, washing the filter paper with 5 ml of glacial acetic acid. Add 5 ml of 3% lead acetate solution in glacial acetic acid, plus 25 ml of anhydrous ethyl alcohol. Centrifuge and decant the supernatant liquid. To the precipitate add 4 ml of 10% sodium bisulphate solution, 10 ml of 1% periodic acid solution, and 10 ml of water. Allow oxidation to take place at room temperature for 20 minutes. Destroy the excess oxidizing agent by addition of powdered sodium bisulphite. Add 4 ml of p-nitrophenylhydrazine reagent and place the centrifuge bottle in boiling water for ten minutes. Filter the solution into a separatory funnel, cool and extract the aqueous layer with 50 ml of ethyl ether. Wash the ether extract with 5 ml of distilled water and pass the washed extract through the packed adsorption column. Wash the column with 30 ml of ethyl ether and discard the washings. Elute with 20 ml of anhydrous ethyl alcohol. Dissolve about 0.1 g of solid sodium hydroxide in the eluate. A brilliant pink to red coloration is obtained if tartrates were present in the original sample. The color is stable upon dilution with an equal quantity of water.

DISCUSSION

Table 1 shows the results of the test on a number of wines and fruit acids. The wines utilized in these tests were made by Peter Valaer in the laboratory of the Alcohol Tax Unit, Washington, D. C. No question exists as to their authenticity or chance contamination, as might be the case

TABLE 1.—*Results of color tests*

SAMPLE	COLOR (ALKALINE ELUATE)	TARTRATES
Grape:		
Scuppernong	Red	Positive
Muscat	Red	Positive
Concord	Red	Positive
Muscadine	Red	Positive
Raisin	Red	Positive
Blackberry	Colorless—Faint Yellow	Negative
Strawberry	Colorless—Faint Yellow	Negative
Loganberry	Colorless—Faint Yellow	Negative
Orange	Colorless—Faint Yellow	Negative
Rhubarb	Colorless—Faint Yellow	Negative
Peach	Colorless—Faint Yellow	Negative
Honey	Colorless—Faint Yellow	Negative
Apple (Often no precipitate with 5% lead acetate)	Colorless—Faint Yellow	Negative
Elderberry (Often no precipitate with 5% lead acetate)	Colorless—Faint Yellow	Negative
Cherry	Colorless—Faint Yellow	Negative
10% Grape—90% Blackberry	Red	Positive
1% Grape—99% Blackberry	Pink	Positive
Fruit Acids: (10 mg. in 50 ml. of aqueous solution)		
Tartaric Acid, 10 mg.	Red	Positive
Citric Acid, 10 mg.	Colorless	Negative
Malic Acid, 10 mg. (No precipitate with lead acetate)		
Maleic Acid, 10 mg. (No precipitate with lead acetate)		
Isocitric Acid, 10 mg.	Colorless	Negative
Aconitic Acid, 10 mg.	Colorless	Negative

when fruit or berry juices are fermented in vats previously used to ferment grape juice. The organic acids used were of Reagent Grade.

ADSORBENTS

A number of adsorbents have been found useful for packing the adsorption column.

Powdered glass adsorbs the p-nitrophenylhydrazone of glyoxylic acid excellently from ethereal solution, offers ease of elution with anhydrous alcohol, and the solutions pass through the column rather quickly. Suction may be used with this adsorbent to speed the passage of the liquids. Powdered glass purchased from Eimer and Amend, ground glass prepared from whiskey bottles and glass wool "Corning" Brand Fibre Glass No. 008 were used with equally good results. The glass wool, however, does not give as uniform packing as the powdered glass.

Calcium carbonate, reagent grade, is a good adsorbent and may be used alone or in combination with filtercel to speed the process of adsorption and elution. Exhaustive washing with ether must be avoided as the p-nitrophenylhydrazone of glyoxylic acid is gradually eluted thereby.

Activated alumina is too powerful an adsorbent and the same may be said of calcium hydroxide, though the latter may be used with some modification of the procedure.

Anyhydrous potassium carbonate provides a good column packing.

For the separation of a mixture of p-nitrophenylhydrazones, U.S.P talc is an excellent adsorbent. Roberts and Green (6) separated the 2,4-dinitrophenylhydrazones of low molecular weight aliphatic aldehydes by chromatographic adsorption on a column of silicic acid-Super Cel. When using a column of talc, the p-nitrophenylhydrazones are adsorbed from a solution of ethyl ether-petroleum ether. The chromatogram is developed by increasingly greater concentrations of ethyl ether. For tartrate detection by this method, talc is not as suitable as powdered glass or calcium carbonate, as it must be handled with some skill to effect separations without complete elution of the desired p-nitrophenylhydrazone of glyoxylic acid.

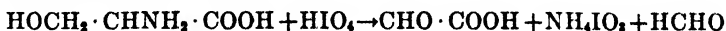
INTERFERING SUBSTANCES

α,β -Dihydroxy acids will give positive tests under the prescribed conditions but no such acids, other than tartaric, have been reported in wines.

Polyhydroxy compounds (such as sugars, glycerine, proteins, dextrans, caramel and coloring materials) are removed by treatment with activated carbon and washing of the lead precipitate. Such compounds, if not removed, are oxidized to a variety of products which will react with p-nitrophenylhydrazine to produce p-nitrophenylhydrazones and osazones, including the p-nitrophenylhydrazone of glyoxylic acid (7). Alkaline solutions of such compounds produce powerfully colored solutions.

Tannins are very nearly quantitatively removed by the charcoal treatment. Traces remaining which form p-nitrophenylhydrazones, seem to be held on the adsorbent more firmly than the glyoxylic acid derivative, and thus do not interfere.

Amino acids from protein degradation appear the most difficult interfering substances to remove. Martin and Synge (8) have applied periodic acid oxidations to some hydroxyamino-acids of protein hydrolyzates. Any amino acid containing adjacent $-\text{NH}_2$ and $-\text{OH}$ groups is oxidized by periodic acid to aldo-acids, and as such will follow the oxidation product of tartaric acid throughout the test. Serine, for example, is oxidized to glyoxylic acid as follows:



Some p-nitrophenylhydrazones have been produced from wines which

chemically behave very similarly to the glyoxylic acid derivative, but these have been separated therefrom on a column of talc. Dakin and Dudley (9) report the slow conversion of 2-amino and 2-hydroxy acids to ketonic aldehydes.

Pectin degradation products, if not removed, will form aldo- and keto-hydroxy acids upon oxidation, and their p-nitrophenylhydrazones will give positive tests. Apple wines containing large amounts of pectin, and solutions prepared from powdered citrus pectin, have been tested by this method with negative results.

OTHER REAGENTS

In addition to p-nitrophenylhydrazine reagent, a 2,4-dinitrophenylhydrazine reagent has been employed in a similar manner. The 2,4-dinitrophenylhydrazone is more sensitive to hydroxyl ion concentration (10), but the color produced by the glyoxylic acid derivative is not as distinctive as that from the p-nitrophenylhydrazone of glyoxylic acid.

SUMMARY

A satisfactory method is presented for the detection of tartrates in quantities as small as 0.1 mg. per ml. of solution, in the presence of other fruit acids and wine ingredients. Tartaric acid has been found only in grape wine and wines produced from some grape product.

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DETERMINATION OF BORAX IN MIXED FERTILIZERS*

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The present official A.O.A.C. methods for determination of borax in mixed fertilizers have met with general dissatisfaction in recent years. In many instances a sizable fraction of the borax known to have been added has not been found, resulting in unsatisfactory reports by testing laboratories and necessitating "overdosing" with borax by the manufacturer. It is difficult to assign reasons for these errors. The situation has sometimes been confused by the non-uniform mixing of added borax, and oftentimes by the use of two different analytical methods, neither of which gives consistently good results. Usually the method employed has not been designated, so a statistical study of results which have been obtained is not feasible. It is difficult to understand why methods showing good results when first developed some years ago should now seem unreliable. One factor may be that the amounts of borax involved were usually lower in earlier use of the methods, since the primary concern was then with borax contaminated materials rather than with deliberate borax additions. Also the wide range of materials which can be present in fertilizers makes it impossible to be sure all conditions have been encountered in preliminary testing of new methods.

The A.O.A.C. methods in current use are known as the "water soluble" and "acid soluble" methods. The "water soluble" method (2) makes use of solution in water or dilute hydrochloric acid and precipitation of interfering substances by treatment with barium chloride and barium hydroxide. The "acid soluble" method (1) involves treatment of sample with phosphoric acid and distillation with methanol to remove boron from interfering substances.

It was our belief that under present conditions no useful purpose is served by having a method for "water soluble" boron. Slightly soluble borates which might form on storage of mixtures should be decomposed, dissolved and included in the analytical results, since these represent some of the added borax and presumably are essentially available for plant use. Accordingly, all work on revised methods was designed to insure solution of all borates, and no statement of "water soluble" or "acid soluble" etc., is included in the names of the methods.

It was also our belief that methods involving precipitation removal of interfering substances could be developed to adequate accuracy and precision for the purposes involved, and that such methods are more suited to intermittent use in laboratories principally concerned with other types of analyses. The methods being reported are of the precipitation

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removal type, and have been shown to be adequate in all cases tried. By adequate we mean accuracy within less than 0.10% borax (or 2 pounds borax per ton fertilizer) and precision substantially better than that ($\pm 0.03\%$ average deviation). Errors of this amount do not seem serious with borax added in amounts ranging from 10–100 pounds per ton, though better accuracy would of course be desirable. It is possible that methods involving methyl borate distillation could ultimately be developed to give greater accuracy and precision.

PRELIMINARY STUDIES

There are two major problems involved in determination of boron oxide content of fertilizer by methods involving precipitation removal of interfering substances. Unless both of these problems are satisfactorily solved there can be no hope of accurate results. These problems are:

- (a) Complete solution of boron oxide in the sample.
- (b) Complete removal of interfering substances (mainly phosphate) by precipitation without serious loss of boron oxide. (If the phosphate ion is not removed, the titrations for boron oxide will of course be high.)

These two problems are described in more detail in the following:

(a) *Complete solution of boron oxide* is not difficult to accomplish. Though water is adequate if all boron oxide is present as borax it was felt the possibility of forming slightly soluble calcium borates or borophosphates on storage prevents its general reliability. Therefore any method should insure solution of such borates, even though by so doing more interfering substances (phosphate mainly) are also put in solution. Several means for dissolving slightly soluble borates suggest themselves, and we have confined ourselves to use of (1) dilute hydrochloric acid or (2) sodium carbonate solutions.

(b) *Complete removal of interfering substances by precipitation without boron oxide loss* is more difficult. Iron and aluminum are readily precipitated in neutral solution, and phosphate is readily removed by barium hydroxide. However, when much phosphate is precipitated by barium hydroxide as in the present A.O.A.C. method there is much danger of loss of boron oxide. Our earliest work showed that if the A.O.A.C. water soluble method (for inorganic compounds) is used as directed on some fertilizers containing carbonates (slight excess of hydrochloric acid employed in dissolving) the results are very unsatisfactory. Much phosphate is dissolved and a very sticky precipitate which holds boron oxide is obtained when barium hydroxide is added. The amount of boron oxide lost seems to vary widely and erratically, and is not solely dependent on the amount of phosphate present in the solution treated. In fact simultaneous presence of much calcium ion (which is common) seems to greatly increase the difficulty.

It thus appeared that the major problem was to find some means for precipitating all the phosphate without these boron oxide losses. Attempts

to accomplish this by use of barium chloride and limited amounts of barium hydroxide or ammonium hydroxide so that the final pH was not so high, were not successful. Lower pH tended to decrease boron oxide loss but phosphate was not so adequately removed. Trial of several other possibilities uncovered one promising method, consisting of the addition of excess solid barium carbonate to an acid solution containing considerable barium chloride. The use of barium carbonate alone added to acid extracts from borate ores to precipitate impurities before boron oxide titration has been common (3). It is a very convenient way of establishing a pH near 7 without tedious adjustment (excess reagent merely remains undissolved). The pH obtained does not appear high enough to result in precipitation of all phosphoric acid, and trials demonstrated that using barium carbonate alone resulted in small amounts of phosphate left in solution. If however a considerable concentration of barium ion was already present in the solution substantially all the phosphate was precipitated. Conceivably this barium ion could be obtained by using much acid and barium carbonate, but we have preferred to add a measured amount of barium chloride to the solution (in excess of amount reacting with sulphates).

This technique which we might call the barium chloride-barium carbonate method was first tried on solutions obtained by digesting samples in dilute hydrochloric acid. On some samples very good results were obtained but on others an unexpected and curious disadvantage was discovered. Large amounts of magnesium ion in the solution being treated in some way to prevent the barium carbonate acid reaction from proceeding to completion. In extreme cases the barium carbonate will not react sufficiently to make the solution basic to methyl red (pH 5-6). In other cases the indicator changes but the pH is insufficient to quite remove the phosphate. The reason for this behavior is not known, but since dolomite is widely used in fertilizers, dissolving methods must be employed which minimize magnesium content of the solution. Use of a sodium carbonate solution is proposed. The methods required are somewhat more tedious than would be possible if the magnesium interference did not exist.

All precipitation methods tend to result in a final solution for titration which has a rather high salt content. This tends to produce a less satisfactory end point. Variations in salt concentration may also result in a variable blank which may be responsible for somewhat lower precision than might be desired. However, in general, results are believed adequate for purposes in mind.

When organic matter is present in the sample being analyzed the method which must be used is analogous but also includes a carbonizing step to destroy interfering organic matter. Also physical properties of the sample dictate some variations in manipulation, so the method has been written out in full for such cases.

METHODS

Reagents—Same as in present A.O.A.C. Method.

I. *Materials Free From Organic Matter*

Weigh a 5 g sample into a 250 ml beaker, add 40 ml of 5% Na_2CO_3 soln, 5 drops phenolphthalein (2.10d), and ca 35 ml of water. Boil, adding water as needed to keep volume at about 70–80 ml. Boil at least 15 min., keeping color of phenolphthalein decidedly red by adding more Na_2CO_3 soln if required. If NH_3 is present boil $\frac{1}{2}$ hour after the smell of NH_3 has disappeared. Filter the hot soln and wash solids well with hot water. Add a few drops of methyl red to the soln in a 250 ml beaker, and boil until the volume is about 80–100 ml, acidify cautiously with conc. HCl (pink to methyl red), and boil a few minutes longer. Add slowly 10% $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ soln, until it is evident that sulfates have all precipitated (probably 20–30 ml), then add 10 ml in excess. Heat mixture a few minutes near boiling and cautiously add powdered BaCO_3 with stirring until the soln shows the yellow of methyl red. Add a small excess and boil a few minutes. If enough BaCO_3 has been added a very persistent foam is obtained. Filter into a 500 ml Erlenmeyer flask, wash solids well with hot water. Cautiously acidify the filtrate with HCl to the pink of methyl red and boil a few minutes to expel CO_2 . Cool, and neutralize exactly, using 0.5 N NaOH at first and finally 0.1 N NaOH , reacidifying with 0.1 N HCl if at first the neutral point (orange) is exceeded. Add several g of neutral mannitol, a few tenths of a ml phenolphthalein (2.10d) and titrate with 0.1 N NaOH to the first appearance of orange color due to the phenolphthalein red. (Color after mannitol addition is red, goes through yellow, to orange and red again as alkali is added.) Add a little more mannitol and if the phenolphthalein color disappears titrate carefully until it reappears. Repeat this procedure until addition of mannitol has no further action on the color. Note volume of 0.1 N NaOH used. Run a blank using the same amount of reagents and subtract the amount of 0.1 N NaOH required for the blank from the gross titration. Calculate the borax content of the sample.

$$\% \text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O} = \frac{\text{net ml } 0.1 \text{ } N \text{ NaOH} \times 0.954}{\text{g sample}}$$

II. *Materials Containing Organic Matter*

Weigh a 5 g sample into a 250 ml beaker, add 40 ml of 5% Na_2CO_3 soln, 5 drops of phenolphthalein, and about 35 ml of water. Boil the mixture gently for a few minutes. (Care is required to prevent frothing over.) If mixture is not alkaline to phenolphthalein (external spot test if necessary) add more Na_2CO_3 soln and heat a very short time longer. Filter the hot soln and wash solids well with hot water. Place filtrate and washings in a 250 ml beaker, boil until volume is ca 80 ml, acidify cautiously with HCl until acid to methyl red (external spot test may be necessary), and boil a few minutes longer. Add 10% $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ soln slowly until sulfates are precipitated (perhaps 10–20 ml) and then 10 ml in excess. Heat mixture near boiling and add enough BaCO_3 to make alkaline to methyl red (external spot test may be required) followed by a small excess. Boil briefly and filter the hot soln, washing solids well with hot water. Put filtrate and washings in a large casserole, add $\text{Ba}(\text{OH})_2$ solid to keep alkaline to phenolphthalein, and evaporate to dryness. When dry, carbonize the organic matter thoroly just below redness (500–550°C. in muffle furnace preferably). When cool, add 50 ml hot water, methyl red indicator, and just enough HCl to make acid; heat to dissolve. When soln is complete and hot soln is acid, add excess solid BaCO_3 , boil briefly, and filter into a 500 ml Erlenmeyer flask, wash solids well with hot water. Cautiously acidify the filtrate with HCl and boil a

few minutes to expel CO_2 . Cool and neutralize exactly using 0.5 *N* NaOH at first and finally 0.1 *N* NaOH, reacidifying with 0.1 *N* HCl if at first the neutral point (orange) is exceeded. Add several grams of neutral mannitol, a few tenths of a ml phenolphthalein (2.10d) and titrate with 0.1 *N* NaOH to the first appearance of the phenolphthalein red. Add a little more mannitol and if the phenolphthalein color disappears titrate carefully until it reappears. Repeat this procedure until addition of mannitol has no further action on the color. Note volume of 0.1 *N* NaOH used. Run a blank using the same amount of reagents and subtract the amount of 0.1 *N* NaOH required for the blank from the gross titration. Calculate the borax content of the sample.

$$\% \text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O} = \frac{\text{net ml 0.1 N NaOH} \times 0.954}{\text{g sample}}$$

NOTES: (1) In this laboratory it is preferred to use suction with a platinum cone for all filtrations.

(2) Throughout these procedures care should be exercised to avoid more reagents than necessary. Over additions of acid and alkali build up the salt content of final solution with some effect on accuracy of titration.

(3) In this laboratory methylene blue is often added to the solution during titration as the color changes (purple to green to purple) are somewhat easier to observe. The amount to add can be found by trial, and should not be so great that it tends to mask the indicator color changes.

SAMPLES USED

Fertilizer No. 1—*F. S. Royster Guano Company 0-12-12 Fertilizer*, said to contain KCl, superphosphate, and about 4% borax. Seemed to contain dolomite also.

Fertilizer No. 2—*Magruder Check Sample No. 9, 2-12-2 Fertilizer*, prepared by F. S. Royster Guano Company. Borax content 4.40% (88 pounds per ton).

Fertilizer No. 4—*Laboratory Mixture as Follows:*

19% Ammonium Sulfate (Ford Motor Company)	
16% KCl	} (Supplied by F. S. Royster Guano Co., Norfolk, Va.)
35% Superphosphate	
30% Pulverized Dolomite (U. S. Lime Products Corp., Los Angeles, California)	

Fertilizer No. 5—*Laboratory Mixture as Follows:*

19% Ammonium Sulfate (Ford Motor Company)	
16% KCl	} (Supplied by F. S. Royster Guano Co., Norfolk, Va.)
40% Superphosphate	
25% Ground Limestone	

Fertilizer No. 6—*Made by Adding 10% Royster Superphosphate to "Red Star Gro-Master V" (4-10-2)*. Said to contain blood meal, bone meal, cottonseed meal, superphosphate, KCl and K_2SO_4 . Manufactured by Downey Fertilizer Company, Downey, California.

RESULTS

Test results on mixtures using four different inorganic fertilizers and one organic fertilizer are shown in Table 1. In most cases comparative results

by the A.O.A.C. water soluble method were obtained and are shown. The data include results obtained by three different operators.

Where not otherwise indicated, the borax additions were made just before analysis. In all such cases the agreement with the amount added is good by the new methods, and generally very poor by the A.O.A.C. water soluble method.

Some samples with borax were stored six months at 35–40°C. before

TABLE 1.—*Test results*

FERTILIZER USED, NO.	% BORAX ADDED	SPECIAL TREATMENT	RESULTS BY A.O.A.C. WATER SOLUBLE METHODS			RESULTS BY PROPOSED METHODS		
			% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND	% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND
1	0	—	1.90			3.45	0.00	3.45
			1.47			3.45	0.00	
			1.60	?	?	3.40	−0.05	
			2.10			3.45	0.00	
			1.90			3.45	0.00	
			2.03			3.40	−0.05	
						3.47	+0.02	
						3.48	+0.03	
						3.48	+0.03	
	2.00	—	—			5.42	−0.03	5.45
						5.42	−0.03	
						5.43	−0.02	
						5.50	+0.05	
						5.48	+0.03	
						5.47	+0.02	
2	0	Contains 4.40 % Borax				4.28	−0.03	4.31
						4.32	+0.01	
						4.33	+0.02	
4	0	—	0.03		0.04	0.07		0.06
			0.04			0.05		
						0.06		
	0.50	—				0.54	−0.03	0.57
						0.60	+0.03	
						0.58	+0.01	
	2.00	—	0.98	+0.05	0.93	2.03	−0.03	2.06
			0.90	−0.00		2.01	−0.05	
			0.85	−0.08		2.04	−0.02	
			1.01	+0.08		2.06	0.00	
						2.08	+0.02	
						2.05	−0.01	
						2.11	+0.05	

TABLE 1—(continued)

FERTILIZER USED, NO.	% BORAX ADDED	SPECIAL TREATMENT	RESULTS BY A.O.A.C. WATER SOLUBLE METHODS			RESULTS BY PROPOSED METHODS		
			% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND	% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND
4	0	Stored 6 months 35-40°C.	0.04		0.04	0.07	+0.03	0.04
			0.05			0.03	-0.01	
						0.02	-0.02	
	0.50	Stored 6 months 35-40°C.	0.64	+0.28	0.36	0.59	+0.02	0.57
			0.25	-0.14		0.54	-0.03	
			0.20	-0.16		0.59	+0.02	
	1.00	Stored 6 months 35-40°C.	0.34	+0.03	0.31	1.07	-0.03	1.10
			0.38	+0.07		1.09	-0.01	
			0.22	-0.09		1.13	+0.03	
	2.00	Stored 6 months 35-40°C.	0.79	-0.02	0.81	2.19	+0.06	2.13
			0.78	-0.03		2.12	-0.01	
			0.85	+0.04		2.08	-0.05	
	6.00	Stored 6 months 35-40°C.	3.64	+0.24	3.40	6.50	0.00	6.50
			3.24	-0.16		6.55	+0.05	
			3.32	-0.08		6.46	-0.04	
5	0	—	—	—	—	0.03		0.05
						0.06		
	1.00	—	—	—	—	0.99	-0.05	1.04
						1.08	+0.04	
						0.99	-0.05	
	2.00	—	—	—	—	1.08	+0.04	
	4.00	—	—	—	—	2.04	+0.01	2.03
						1.99	-0.04	
						2.06	+0.03	
	0	Stored 6 months 35-40°C.	0.16	+0.02	0.14	2.02	-0.01	
			0.09	-0.05		4.00	+0.01	3.99
			0.16	+0.02		3.99	0.00	
	0	Stored 6 months 35-40°C.				4.03	+0.04	
						3.95	-0.04	
	0	Stored 6 months 35-40°C.	0.16	+0.02	0.14	0.11	+0.01	0.10
			0.09	-0.05		0.11	+0.01	
			0.16	+0.02		0.07	-0.03	

TABLE 1—(continued)

FERTILIZER USED, NO.	% BORAX ADDED	SPECIAL TREATMENT	RESULTS BY A.O.A.C. WATER SOLUBLE METHODS			RESULTS BY PROPOSED METHODS		
			% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND	% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND
5	0.50	Stored 6 months 35-40°C.	0.27	0.00	0.27	0.76	+0.17	0.59
			0.25	-0.02		0.60	+0.00	
			0.28	+0.01		0.57	-0.02	
						0.63	+0.04	
						0.44	-0.15	
						0.51	-0.08	
	1.00	Stored 6 months 35-40°C.	0.75	+0.18	0.57	1.05	-0.02	1.07
			0.46	-0.11		1.03	-0.04	
			0.51	-0.06		1.14	+0.07	
	2.00	Stored 6 months 35-40°C.	1.03	+0.27	0.76	2.16	+0.07	2.09
			0.93	+0.17		2.06	-0.03	
			0.33	-0.43		2.06	-0.03	
	6.00	Stored 6 months 35-40°C.	4.42	+0.64	3.78	6.26	-0.10	6.36
			4.15	+0.37		6.35	-0.01	
			2.78	-1.00		6.60	+0.24	
						6.36	0.00	
						6.21	-0.10	
6 (Organic)	0.00	—	0.05	-0.02	0.07	0.07	0.00	0.07
			0.07	0.00		0.06	-0.01	
			0.08	+0.01		0.08	+0.01	
	1.00	—	0.95	-0.04	0.99	—	—	—
			1.00	+0.01				
			1.02	+0.03				
	2.00	—	—	—	—	2.00	-0.05	2.05
						2.02	-0.03	
						2.03	-0.02	
						2.04	-0.01	
						2.06	+0.01	
						2.06	+0.01	
						2.08	+0.03	
						2.09	+0.04	
						2.07	+0.02	
	4.00	—	3.89	-0.06	3.95	4.10	-0.02	4.12
			3.94	-0.01		4.10	-0.02	
			4.03	+0.08		4.13	+0.01	
						4.14	+0.02	
						4.14	+0.02	

analysis. The results by the proposed method are somewhat higher than expected (presumably due to water loss from samples) but show satisfactory reproducibility. Results by the A.O.A.C. method were erratic and low in all cases (HCl was used since carbonates were present and this may have increased difficulty with the method.)

The average deviation of all results shown by the proposed methods is $\pm 0.03\%$ borax which seems entirely satisfactory.

The result obtained (4.30%) by the new method on the Magruder Check Sample is close to its reported borax content (4.40%).

SUMMARY

Methods for the determination of borax in mixed fertilizers which involve dissolving in sodium carbonate solution and removal of interferences by barium chloride and barium carbonate addition to the acidified extract have been developed. The procedures have been tried on a number of samples and found reasonably accurate and precise. Analysis of the same samples by the A.O.A.C. "water soluble" methods were generally very unsatisfactory.

ACKNOWLEDGEMENT

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STUDY OF THE ENZYMATIC OXIDATION OF THIOUREA IN FROZEN PEACHES

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INTRODUCTION

Early in 1946, the author obtained incomplete recoveries of known amounts of thiourea added to frozen fresh peaches, although good recoveries had been obtained on orange juice by a similar method of analysis (1). Good recoveries could be obtained if the frozen peaches with added thiourea were immersed immediately in boiling water or in a solution of sodium sulfite. These findings led to the conclusion that the enzyme systems of the peach destroy the thiourea. The nature of the reaction between

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the enzyme systems and the thiourea was then studied, and the results of the investigations are reported herein.

HISTORICAL

Investigators (2, 3, 4, 5, 6) have shown that the oxidation of thiourea with chemical oxidizing agents takes place in at least four stages, depending on the agents and the conditions of oxidation. The following successive oxidation products have been identified:

First. Formamidine disulfide salts

Second. Formamidine sulfinic acid (Dioxide of thiourea).

Third. Formamidine sulfonic acid (Trioxide of thiourea).

Fourth. Urea and sulfuric acid.

VARIABLE ACTIVITY OF THE OXIDIZING SYSTEMS OF PEACHES ON THIOUREA

The frozen peaches were cut into sections and a sample of 200–400 grams, consisting of alternate sections, was weighed in a beaker. To this sample was added one-half its weight of a water solution containing a known quantity of thiourea. From the remaining alternate sections a control sample of equal weight was prepared to which was added one half its weight of water containing no thiourea. The cold material was then thoroughly comminuted in a Waring blender and allowed to stand for some time at room temperature to permit the oxidizing reaction to proceed.

The samples to which were added varying amounts of thiourea and which had stood for varying lengths of time, were examined for residual thiourea by a published (1) procedure. A wide variation was found in the activity of the oxidizing systems of different peach samples. Many samples did not destroy thiourea in amounts greater than 25 or 30 p.p.m., and quantities added in excess of this remained unchanged, or were oxidized extremely slowly. Other samples were found to oxidize 80–100 p.p.m. of added thiourea, while a few were capable of readily destroying 130–140 p.p.m. The latter peaches were chiefly used in the search for formamidine disulfide salts and other oxidation products of thiourea as described below.

Search for sulfate and urea.—As stated above, sulfuric acid and urea may be final products of the oxidation of thiourea. In order to determine if the sulfate normally occurring in peaches can be uniformly removed by an aqueous extraction, several sulfate analyses were made. The results showed that it was not uniformly extracted. Therefore, any sulfate which might be formed from thiourea could not be accurately measured and no further effort was made to use sulfate as an index of the progress of oxidation of the added thiourea.

In order to establish definitely whether the oxidation of thiourea in peaches proceeds to the urea stage, tests for urea were made by various methods. The xanthidrol method (7) was modified so that it could be applied to the peach solutions after (a) clarification with ethyl acetate or ether, (b) precipitation with acetone, and (c) evaporation of the acetone. Fifteen ml portions of the solutions were usually taken, and the quantity of reagents was correspondingly increased. The amounts of thiourea added were 79, 110, and 178 p.p.m. Controls with and without added urea were also run. The test gave only an opalescent solution on samples, with or without added thiourea, indicating little or no urea present. The control with 0.5 mg added urea gave a positive test in 10–15 minutes.

Preliminary tests for urea by the urease method were inconclusive, but the effects of the sugar and salt concentration on the urease were not fully appreciated at that time. Preparatory to applying the method to frozen peaches with their natural content of sugars, acid, etc., a study of these effects was conducted and some of the results are given in Table 1.

TABLE 1.—*Urease activity in various solutions of sugars and salts, using $\frac{1}{2}$ grain of urease per determination*

TEST NO.	COMPOSITION OF AQUEOUS TEST SOLUTION	UREA ADDED	TOTAL VOLUME OF SOLUTION	TIME OF UREASE ACTION	ALKALI ADDED FOR DISTILLATION NH_3	0.02 N HCl CONSUMED
		mg		minutes		ml
1	Water only	10	100	30	13 ml 15 % NaOH	16.4
2	10 % sucrose	10	100	40	13 ml 15 % NaOH	13.5
3	8 % sucrose 2 % dextrose	10	100	40	2 g. MgO	12.21
4	6 % sucrose 1.5 % dextrose	10	100	90	2 g. MgO	16.17
5	2.5 % dextrose 5 % sucrose 0.33 % malic acid NaOH added to pH 6.9	5	120	105	2 g. MgO	6.01
6	Water and urease only	0	100	45	2 g. MgO	0.32

Tests 1, 2, 3, and 4 show that 10 per cent, but not 7.5 per cent, of sugars reduces the activity of the urease. Test 5 shows that sodium malate formed by the neutralization of malic acid with sodium hydroxide also reduces the activity of the urease.

Finally, a series of tests was conducted where there could be no possibility of the inhibition of urease by sugar, salt, etc., and where the urease was acting at the optimum pH.

A carton of commercial frozen peaches containing no thiourea was divided into two portions as in previous work, and 100 p.p.m. of thiourea were added to one portion. Both portions were held at room temperature until the thiourea portion gave a negative Grote test. (The test was also negative on the control portion.) Fifty gram samples of each portion were diluted to 220 ml and distilled with magnesium oxide instead of sodium hydroxide to minimize breakdown of organic matter. The distillate from the control had a titer of 1.75 ml of 0.02 *N* HCl; that from the thiourea portions had a titer of 2.0 ml. The experiment was repeated on similar pair of samples which had been treated with urease at pH 6.8–6.9 before distillation. The titers of the distillates were 2.5 and 2.8 ml. respectively. Thus any difference in the ammonia content between control and thiourea samples was no greater with the urease treatment than without it. This furnishes definite evidence that urea is not present in frozen peaches as an oxidation product of thiourea.

Search for formamidine disulfide cleavage products.—The formamidine disulfide salts, particularly the salts of organic acids, are unstable and suffer disintegration by molecular cleavage. At least two disintegration patterns have been suggested in the literature. Boeseken's mechanism for the breakdown would produce free sulfur, sulfocyanic acid, cyanamide, and ammonia. (2) Werner claims that the products of the molecular disintegration are thiourea, free sulfur, and cyanamide. (4) To determine which if any, of these products were formed by cleavage of the disulfide salts in solution, two salts (sulfate and hydrochloride) of formamidine disulfide were prepared (2) The reactions of these salts were studied, and tests were made for the products of decomposition. The disulfide salts gave a positive reaction with Grote's reagent similar to thiourea. This is in accord with Grote's (8) original findings that compounds having the S—S linkage give a positive reaction with the reagent.

Aqueous solutions of the prepared salts after standing some time were examined for sulfocyanic acid and cyanamide. Tests for sulfocyanic acid proved negative on the solution of the sulfuric acid salt and only a very faint positive test was obtained on the solution of the hydrochloride salt. The test (see below) for cyanamide showed an abundance of this degradation product to be present in the solution from both of the salts of the disulfide. The test used (bright yellow amorphous precipitate in ammoniacal silver nitrate solution) is characteristic of cyanamide. (2)

The formamidine disulfide salt solution continued to give a strong positive Grote reaction on further standing. These findings suggest that the breakdown is probably along the Werner pattern. The breakdown in this manner would reproduce one-half of the original thiourea. In peaches this

reformed thiourea would in turn be acted on by the enzyme systems of the peaches. Eventually, according to this theory, all residual thiourea in peaches would be converted to cyanamide and free sulfur.

In order not to overlook any possible cleavage products, the fruit samples, with and without added thiourea, were examined for ammonia, sulfocyanic acid, and cyanamide. Tests for sulfocyanic acid were negative on all samples. Results of ammonia tests on thiourea-treated samples were not significantly different from those of the controls.

The tests for cyanamide on all of the fruit samples, were negative. As a check on the method, cyanamide was prepared in the laboratory and a small amount (2-3 mg per cent) was added to control samples. Strong positive cyanamide reactions were obtained.

The test for cyanamide was made by the following method devised by the author and based on some of the known properties of cyanamide.

METHOD FOR DETECTION OF CYANAMIDE

Place 100 ml of sample soln in a 500 ml separatory funnel. Extract liquid by shaking with 300 ml of ether (washed to remove alcohol), allow layers to separate and draw off aqueous layer into second 500 ml separatory funnel; then pour off the ether layer thru filter into a distillation flask. Rinse first funnel with 300 ml of washed ether and run into second funnel. Extract sample as before, and repeat extraction a third time in similar manner. Combine three ether extracts in distillation apparatus and distil off most of ether until only about 30-50 ml remain. Transfer remainder to a 250 ml beaker marked at 40 ml, rinsing the distillation flask 2 or 3 times with 10-15 ml portions of ether. Evaporate to 40 ml mark and pour ethereal liquid into 125 ml separatory funnel. Add 10 ml of water to beaker, rinse, and pour into funnel containing ether extract.

Extract ether solution with water and repeat with a second 10 ml portion of water. Combine water extracts and test 5 ml portion with Ca 1 ml of weakly ammoniacal soln of AgNO₃. An immediate bright yellow amorphous precipitate indicates cyanamide. For additional confirmation, determine nitrogen by micro-kjeldahl in the remaining 15 ml of aqueous extraction of ether extract.

According to the literature (9), cyanamide hydrolyzes to urea; but this hydrolysis proceeds very slowly, if at all, at room temperature in neutral or weak acid solution, as shown by the fact that dilute solutions remained for weeks in the laboratory with little if any noticeable change in the strength of the positive test.

It is therefore apparent that the oxidation of thiourea proceeds rapidly through and beyond the disulfide stage and also that the disulfide does not break down into molecular cleavage products.

SEARCH FOR OXIDES OF THIOUREA

Since previous experiments had shown that the thiourea oxidation product produced by the fruit enzymes is neither formamidine disulfide nor urea, it seemed reasonable to suppose that it must be an oxide, intermediate between these two degradation products.

The following test showed that the oxidation product is one or both of the oxides of thiourea:

In establishing absence of urea, it had been shown that little if any ammonia is formed when thiourea is acted on by the enzyme systems of peaches. Previous work has shown that hydrogen peroxide in alkaline solution oxidizes thiourea or its intermediate oxidation products to urea and sulfuric acid. An experiment was conducted in which alternate sections of the same lot of frozen peaches, with and without added thiourea, were allowed to stand until the test for thiourea on the treated sample was negative. The resulting sample solution was then oxidized with hydrogen peroxide in alkaline sodium hydroxide solution, adjusted to pH 6.9, treated with urease and subsequently distilled over magnesium oxide. A 50 gram sample containing no added thiourea showed a titer of 2.85 ml of 0.02 ammonium chloride, and a 50 gram sample containing 100 p.p.m. of added thiourea showed a titer of 6.15 ml. This experiment, together with the previous tests showing absence of urea in the enzymatically oxidized solution, of portions of the same sample, conclusively showed that the increase in ammonia came from the intermediate oxidation products *i.e.*, oxides of thiourea.

Test for dioxide of thiourea.—Boeseken (2, 3) states that, in alkaline solution, the dioxide of thiourea is a very powerful reducing substance; it reduces solutions of numerous metallic salts to the free metal with the application of but little heat; that solutions of the dioxide are unstable on standing, dilute solutions becoming acid due to the formation of sulfurous acid; that alkaline solutions sodium hydroxide of the dioxide break down with the formation of cyanamide.

The dioxide of thiourea was prepared following the method of Barnett (5). No detectable amount of sulfurous acid or cyanamide was found in very dilute solutions of this chemical, comparable in strength to those which would be obtained in the examination of frozen peaches. Reactions of the dioxide with metallic salts were tried but could not be applied effectively under the conditions existing in the peach solutions. Although these tests are not sensitive enough to detect the decomposition products of the dioxide in the quantities in which they would be formed in peaches from added thiourea, it is believed, however, that because of its strong reducing properties, no significant amount of the dioxide would exist as such in the peaches for any appreciable length of time.

Test for trioxide of thiourea.—The literature (3) states that the trioxide of thiourea, when treated with barium hydroxide, breaks down immediately to give barium sulfite and cyanamide. This was confirmed when a 10 mg per cent solution of the trioxide (prepared according to Boeseken's directions (3) gave an immediate precipitate with barium hydroxide. This precipitate was soluble in hydrochloric acid with a strong odor of sulphur dioxide. The filtrate gave the bright yellow precipitate with ammoniacal silver nitrate characteristic of cyanamid.

As in the other tests for urea, divided samples of frozen peaches were prepared, and both the control and the thiourea portions were extracted

with ether as in the tests for disulfide. The ether extracts were tested for cyanamid with ammoniacal silver nitrate both before and after treatment with barium hydroxide. Nitrogen determinations were also made on the acidified ether extracts by a micro-Kjeldahl method. The results are shown in Table 2.

TABLE 2.—Comparative results

	MODIFIED GROTE TEST	CYANAMID TEST		NITROGEN ml 0.01 N HCl ¹
		BEFORE Ba(OH) ₂	AFTER Ba(OH) ₂	
Control No. 1	Negative	—	Negative	0.23
Control No. 1 +100 p.p.m. thiourea	Negative	Negative	Positive	0.8 ²
Control No. 2	Negative	—	Negative	0.25
Control No. 2 +119 p.p.m. thiourea	42 p.p.m.	Faint Positive	Strong Positive	2.23

¹ 0.05 mg cyanamid contained nitrogen equivalent to 0.91 ml 0.01 N HCl.

² Only about one-fourth of the ether extract used for determination.

It thus appears that the trioxide of thiourea is the principal decomposition product of the enzyme oxidation of thiourea in peaches.

SUMMARY AND CONCLUSIONS

The reactions of thiourea with the enzyme oxidizing systems of frozen peaches were studied. No evidence was obtained of the presence of formamidine disulfide, dioxide of thiourea, or of significant amounts of ammonia or urea, although these substances have been reported in the literature as being products of the oxidation of thiourea, with chemical oxidizing agents. Strong tests were obtained for trioxide of thiourea, and it is concluded that the reaction of thiourea with the oxidizing systems of peaches proceeds rapidly to the stage where the trioxide of thiourea is formed, and that the end product is chiefly, if not entirely, that compound.

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DETERMINATION OF EXCHANGEABLE HYDROGEN AND LIME REQUIREMENT OF SOILS

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Of late the term "exchangeable hydrogen" has assumed the connotation that was formerly assigned to such topics as "soil acidity" and "lime requirement." The problem of quantitative determination of exchangeable hydrogen in soils may, therefore, be viewed as essentially the same problem that has engaged the attention of soil investigators for more than half a century. During the past decade the pH determination by means of the glass electrode has been employed almost universally as an indication of soil acidity. Investigators (2, 5, 16, 18) who had wide experience with this type of determination agree that the pH , without detail consideration of physical and chemical properties of a soil, renders an incomplete picture as to the lime requirement of such soil. There is yet a need for a simple but reliable method for the determination of the quantitative lime requirement, or exchangeable hydrogen at some definite level of saturation.

CONCEPTS OF "LIME REQUIREMENTS"

In 1903 Veitch (24) posed two alternatives as to the fundamental concepts of lime requirement: "To neutralize soil so that it shows faintly alkaline reaction, or to neutralize only to the extent as to give satisfactory yield of acid-sensitive plants, like red clover." He concluded that "A soil faintly alkaline from carbonate of lime furnishes the best conditions for the economic production of crops, and the use of any method for estimating the acidity or the lime requirements of soils should be based on this hypothesis." The question was raised again in 1917 in a report on "the status of the problem of lime requirement" by MacIntire (14), wherein he queries, "By lime requirement do we mean its maximum ability to fix CaO through decomposition of applied lime compounds in the laboratory alone or do we intend that the laboratory practice shall approach at least a correlation to crop response under field practice?" He then proposes a definition of "lime requirement" as "the soil's maximum capacity to decompose $CaCO_3$ in the laboratory." In 1933, under the caption "Criteria of Base Saturation of Soils," Bradfield and Allison (3) proposed the definition of a "base saturated soil" as "one which has reached equilibrium with a surplus of $CaCO_3$ at the partial pressure of CO_2 existing in the atmosphere and at a temperature of $25^\circ C$." The above citations from three outstanding authorities on liming show agreement in principle as to what should constitute a "base saturated" soil, namely, one that will not react further with an excess of calcium carbonate. The definition proposed by Bradfield and Allison is obviously a departure from the concept of exchange neutrality as enunciated by Gedroiz (7)

and by Kelley (12) which is to the effect that a soil is "base-saturated" when it will not release hydrogen ions when in contact with a neutral salt solution. Although Bradfield and Allison recognized this difference, they saw no cogent reason why the additional hydrogen between pH 7 and that of the soil-calcium carbonate equilibrium value should not be included in the calculation of base-absorption capacity of soils. At present there is scarcely any knowledge as to actual quantitative difference in base absorption as arrived at by these two standards for base saturation. The soil-calcium carbonate equilibrium concept has the advantage that it is defined by soil contact with natural liming materials and under natural conditions of contact.

Two distinct problems arise from the adoption of calcium carbonate-equilibration as a standard for a base-saturated soil:

- (1) There must be provided a feasible procedure for attainment of the soil calcium carbonate equilibrium in accordance with the above definition.
- (2) There must be established a correlation between this saturation standard and the degree of saturation necessary for optimum crop production obtained on various types of soils and as recognized in field practice.

LABORATORY PROCEDURES

Because the reaction of the soil with solid calcium carbonate is too slow as a laboratory procedure there had been a number of methods proposed which were thought by their proponents to simulate the soil-calcium carbonate reaction equilibrium, and which would qualify as laboratory procedures. The Veitch calcium hydroxide serial titration procedure (24) is one of the oldest of such methods. It was used extensively in the past and continues in use with some modification in more recent times (6). The calcium bicarbonate titration procedure proposed by MacIntire (13) and a similar procedure by Hutchinson and McLennan (10) have not found wide application. Bradfield and Allison (3) proposed a procedure which depends on the preliminary reaction of the soil with an excess of calcium hydroxide followed by carbon dioxide passage and equilibration with out-door air and determination of the residual carbonate. This method was tested and found by these investigators to represent equilibrium, similar to the system: soil-calcium carbonate-water-air obtained under natural conditions. In a recent review of "chemical methods for estimating lime needs of soils," Peech and Bradfield (18) refer to this method as standard for comparison in the determination of exchangeable hydrogen, as well as in the rapid determination of the lime requirement of soils, but as one not suited for routine work. The serial potentiometric titrations with calcium hydroxide described by Dunn (6) and the titration with calcium hydroxide in the presence of calcium chloride as described by Hardy and Lewis (8) are given in the same paper as types

of the better lime requirement procedures. The Bradfield and Allison *N* ammonium chloride-0.01 *N* ammonium hydroxide titration method, and the ammonium acetate method of Brown (4) in which the exchangeable hydrogen is read from the change in *pH* in relation to ammonium acetate-acetic acid calibration curve, are cited as rapid routine methods. The most serious objection to the two last-mentioned procedures is that the equilibrium *pH* value is a variable quantity and the extent of hydrogen replacement will in each case depend upon the hydrogen content of the soil sample under test. Thus, among the various chemical methods proposed, the Bradfield and Allison calcium carbonate equilibration procedure is the only method that has been subjected to extensive laboratory tests and found to conform to the calcium carbonate-soil equilibration concept of a "base-saturated" soil.

Examination of the data presented by Bradfield and Allison (3, p. 77) on the comparative hydrogen replacement efficiencies of several procedures shows that, on the two soils tested, the barium-acetate method of Parker (17) has produced hydrogen replacements equal to those indicated by the "residual carbonate" method. The significance of these results was not commented upon by Bradfield and Allison, and one is bound to draw the conclusion that the barium-acetate solution of *pH* 7 is capable of replacing hydrogen ions from soils to an extent equal to that of the calcium carbonate equilibration at *pH* 8.4. If that conclusion is correct, it is surprising that so little attention has been given to the barium-acetate procedure with the view of adopting it to the rapid determination of exchangeable hydrogen in soils.

TITRATION OF EXCHANGEABLE HYDROGEN IN Ba- AND Ca- ACETATE

This possibility was investigated with the use of both calcium-acetate and barium-acetate. A portion of the data was presented by us (21) in a report on exchangeable hydrogen at the A.O.A.C. meeting in 1941. It was shown that 1 *N* calcium-acetate solution in contact with soils in the ratio of 1:5 has effected a hydrogen replacement from 50 to 60 per cent of that attained by titration to *pH* 7. The extent of the initial hydrogen replacement varied with time of contact, frequency of agitation, and hydrogen content of charge. A 2-hour continuous agitation has produced a hydrogen replacement from 60 to 70 per cent, and as much as 80 per cent in soils of high organic matter content. A method then proposed consisted of preliminary 2-hour contact with agitation of 10 g. of soil, in duplicate marked A and B, with 50 ml 1 *N* calcium-acetate of *pH* 7 and the determination of the initial hydrogen replacement from the determined *pH* of the system in relation to similar readings on a calcium acetate-acetic acid calibration curve. To A was added twice the quantity of calcium hydroxide that was indicated in the initial replacement, and

TABLE 1.—*Exchangeable hydrogen, as determined by ammonium acetate, by titration with $\text{Ca}(\text{OH})_2$ and $\text{Ba}(\text{OH})_2$ in the respective acetate solutions, in milliequivalents per 100 g of soil*

NO.	SOIL TYPE	BY 1 N NH_4 ACETATE			BY $\text{Ba}(\text{OH})_2$ Ba-ACETATE	BY $\text{Ca}(\text{OH})_2$ Ca-ACETATE	Ca- & Ba-ACETATES AVERAGE	DIFFERENCE Ca & Ba AVERAGE-NH ₄ - ACETATE
		EXCHANGE CAPACITY	REPLACEMENT BASIS	H, BY DIFFERENCE				
1.	Apison silt loam	5.0	2.9	2.1	2.4	2.6	2.5	+ 0.4
2.	Bolton silt loam	12.1	7.7	4.4	5.4	5.1	5.3	+ 0.9
3.	Conasauga silt loam	10.1	7.4	3.7	4.0	4.0	4.0	+ 0.3
4.	Fullerton silt loam	5.9	2.3	3.6	4.2	4.2	4.2	+ 0.6
5.	Hartsells sandy loam	6.1	1.8	4.3	5.2	5.7	5.5	+ 1.2
6.	Sequoia silt loam	8.8	3.1	5.7	6.0	6.4	6.2	+ 0.5
7.	Tellico sandy loam	12.4	5.8	6.6	7.9	7.6	7.8	+ 1.2
8.	Cumberland clay subsoil	10.8	3.6	7.2	5.0	5.2	5.1	- 2.1
9.	Miami sandy loam	13.2	1.0	12.2	14.6			+ 2.4
10.	Wooster silt loam	11.3	4.0	7.3	7.8			+ 0.5
11.	Drummer clay loam	27.4	22.6	4.8	8.3			+ 4.5
12.	Carrington silt loam, B	23.5	21.0	2.5	3.4			+ 0.9
13.	Peat	97.0	21.1	76.0	123.0			+47.0

to B, 20 per cent more than that added to A. After 24 hours' contact with agitation the *pH* values were determined, and recorded on cross section paper that gives *pH* against m.e. of base added, and the base requirement at *pH* 7.0 was read from the intersection of the 2-point line with that of *pH* 7. It may be noted that the rapid ammonium acetate method proposed by Brown (4) follows that part of our titration technique which was designated by us only as "preliminary test." By the Brown procedure this equilibrium hydrogen replacement, however, is taken as the total exchangeable hydrogen.

Our additional work consisted of the determinations of exchangeable hydrogen by ammonium acetate, and by titration with calcium hydroxide and barium hydroxide in the respective acetate solutions on a number of soils. A comparison of these results is given in Table 1. The exchangeable hydrogen values as obtained by barium hydroxide titrations to *pH* 7 in barium acetate were compared further with calcium absorptions in soil systems that contained an excess of 100-mesh calcite.

The data in Table 2 indicate that there is little difference as to the exchangeable hydrogen values, whether obtained by titration in barium-acetate or in calcium-acetate. In every instance the titration to *pH* 7 gave higher results for exchangeable hydrogen than those obtained by the ammonium acetate. The disparities become increasingly high with increase in organic matter content, as is shown by soils Nos. 9, 11, and 13. The barium hydroxide titration in barium-acetate has the advantage over that with calcium hydroxide in calcium-acetate in that the barium hydroxide can be prepared in moderate concentrations and also because the barium-acetate stock solution is less subject to spoilage thru mold growth. The titrations of exchangeable hydrogen by barium hydroxide were carried out in a manner similar to that described (21), except that 5 experimental points were obtained for plotting the titration curves about *pH* 7.

CaCO₃ DECOMPOSITIONS BY SOILS IN RELATION TO TITRATION BY Ba(OH)₂ IN Ba-ACETATE TO *pH* 7

Seven soils and a clay subsoil representative of East Tennessee soil types were mixed with 100-mesh calcite in quantities that approximated 80, 100, 120, 140, and 160 per cent of the base requirement indicated by the titration curve to *pH* 7. These soil mixtures were incubated in an oven at 32°C. and at a moisture content of about 80 per cent of water holding capacities. After 90 days, the soils were air-dried, ground to pass 0.5-mm sieve and the carbonate-carbon dioxide determined on the untreated as well as the calcite treated soils by the steam distillation procedure of Shaw and MacIntire (22). The *pH* values were also determined by the glass electrode in water suspensions of a pasty consistency.

The quantities of calcite applied residual carbonate after 90 days, the

TABLE 2.—Calcium absorptions and resultant pH values of soils treated with calcite, 90 days after addition

NO.	SOIL AND BASE REQUIREMENT AT pH ⁷ *	CaCO ₃ —M.E. PER 100 G			pH	NO.	SOIL AND BASE REQUIREMENT AT pH ⁷ *	CaCO ₃ —M.E. PER 100 G			pH
		APPLIED	RESIDUAL	ABSORBED				APPLIED	RESIDUAL	ABSORBED	
1.	Apison silt loam m.e. 2.5	0 2.24 2.80 3.36 3.92 4.48	.30 .30 .55 .75 1.10 1.45	— 2.2 2.5 2.9 3.1 3.3	6.0 6.9 7.2 7.3 7.5 7.6	5.	Hartsells sandy loam m.e. 5.5	0 4.56 5.70 6.84 7.98 9.12	.35 .35 .60 1.15 1.75 2.75	— 4.5 5.4 6.0 6.6 6.7	5.2 6.9 7.0 7.4 7.5 7.6
2.	Bolton silt loam m.e. 5.3	0 4.00 5.00 6.00 7.00 8.00	.25 .60 .85 1.30 1.50 2.25	— 3.6 4.4 5.0 5.6 6.0	6.2 6.9 7.1 7.2 7.3 7.5	6.	Sequoia silt loam m.e. 6.2	0 5.60 7.00 8.40 9.80 11.20	.25 .55 .85 1.65 2.35 3.75	— 5.2 6.4 7.0 7.7 7.7	5.3 6.9 7.2 7.4 7.6 7.7
3.	Conasauga silt loam m.e. 4.0	0 3.36 4.20 5.04 5.88 6.72	.35 .80 1.20 1.35 1.75 2.40	— 2.9 3.6 4.0 4.5 4.7	6.0 6.6 6.95 7.05 7.14 7.20	7.	Tellico silt loam m.e. 7.8	0 5.92 7.40 8.88 10.36 11.84	.25 .80 1.40 1.65 2.45 3.35	— 5.4 6.3 7.5 8.2 8.7	5.7 6.8 7.0 7.2 7.3 7.4
4.	Fullerton silt loam m.e. 4.2	0 3.52 4.40 5.28 6.16 7.04	.25 .35 .50 .85 1.25 1.95	— 3.4 4.1 4.7 5.2 5.4	5.5 6.5 6.8 6.9 7.2 7.3	8.	Red clay m.e. 5.1	0 4.32 5.40 6.48 7.56 8.64	.20 .55 .60 .90 1.30 2.30	— 4.0 5.0 5.8 6.5 6.5	5.1 6.6 7.0 7.2 7.4 7.5

* Obtained from titrations to pH 7 with Ba (OH)₂ in 1 N Ba-acetate, after 48 hours' contact.

quantities decomposed from each addition, and the resultant pH values are given in Table 2. These data may be examined for two specific points of interest:

First, what pH did the soils attain when the calcium absorption from calcium carbonate was equal to the base-requirement indicated at pH 7 by the laboratory procedure?

Second, how much greater was the Ca-absorption at soil-calcium carbonate equilibration than that at pH 7 (if it may be assumed that such state had been attained in these experiments).

The answer to the first query is found by selecting in the "absorbed" column, Table 2, that value for each soil nearest to "base requirement" indication of that soil and reading the corresponding pH in the next column. Such comparison shows that in 4 instances the pH was 7.2, in 1 instance, 6.8, in 2 instances, 7.0, and in the other instance, 7.1; with a median of 7.1. From the generally observed effects of electrolytes it would be expected that the pH values corresponding to those points of neutralization should have been higher, since the laboratory indications were obtained in the presence of normal barium-acetate concentration, whereas the pH reading in the soil-calcium carbonate systems were made in aqueous suspension. The explanation for this phenomenon probably lies in the compensating factor of biochemical activities such as nitrification and sulfication in the soil-calcium carbonate systems as against the laboratory determinations where such activities are practically nil. The over-all effect of the 100-mesh calcite additions, following the indications by the proposed titration procedure was to produce soils of pH 7 to 7.2 as determined after 90 days' contact.

As to the second point, it is obvious from the same data that the soils continue to react with the excess calcium carbonate beyond pH 7. This is not surprising, since it was shown by investigations at this Station as far back as 1915 that soils continue to decompose calcium carbonate considerably in excess of the Veitch requirement, and it was this phenomenon that led to the distinction between *immediate* and *continued* lime requirement of soils (14, p. 147). The residual calcium carbonate at the 90 day period was in between 1000 to 3000 pounds per 2,000,000 pounds of soils, and the maximum calcium absorptions by the soils, beyond pH 7, ranged from 700 to 1300 pounds. In several instances, namely 5, 6, and 8, of Table 2, the calcium absorptions from the two maximal additions appear to have reached a constant value. However, this may be due to the slow rate of reactivity which failed to produce distinct differences for the variable, but small, excesses of calcite present from the two largest additions. Nevertheless, the average additional calcium absorption from the soils having excess calcium carbonate beyond pH 7 was about 1000 pounds per 2,000,000 pounds of soil, and the highest pH attained was about 7.6. This value is considerably below the pH 8.3

indicated by Bradfield and Allison (3) for calcium-saturated soils in equilibrium with calcium carbonate at the partial carbon dioxide pressure of the atmosphere.

SOIL-CALCITE EQUILIBRATION

Because of the uncertainty attached to the equilibrium status of the 100-mesh calcite treated soils of the preceding experiment, it was thought desirable to prepare soils in which the saturation with calcium

TABLE 3.—Carbonate decompositions by Fullerton and Hartsells soils from increasing additions of $-200+325$ calcite and resultant pH values after 90 days incubation at 20°C

CALCITE ADDED PER 100 g. OF SOIL	FULLERTON				HARTSELLS			
	RESIDUAL CARBONATE	CARBONATE DECOMPOSI- TION	TOTAL BASES*	pH	RESIDUAL CARBONATE	CARBONATE DECOMPOSI- TION	TOTAL BASES*	pH
m.e.	m.e.	m.e.	m.e.		m.e.	m.e.	m.e.	
0	0	0	3.5	4.76	0	0	3.5	4.67
1	0	1.0	4.5	5.10	0	1.0	4.5	4.90
2	0	2.0	5.5	5.43	0	2.0	5.5	5.22
3	0	3.0	6.5	5.70	0	3.0	6.5	5.63
4	0	4.0	7.5	5.98	0	4.0	7.5	6.06
5	0	5.0	8.5	6.37	0	5.0	8.5	6.33
6	0.3	5.7	9.2	6.66	0.3	5.7	9.2	6.70
8	1.0	7.0	10.5	7.14	1.0	7.0	10.5	7.12
10	2.8	7.2	10.7	7.26	2.5	7.5	11.0	7.40
12	4.6	7.4	10.9	7.28	4.3	7.7	11.2	7.46
14	6.6	7.4	10.9	7.30	5.8	8.2	11.7	7.50
16	8.6	7.4	10.9	7.35	8.1	7.9	11.4	7.53
18	10.5	7.5	11.0	7.32	10.0	8.0	11.5	7.55
20	12.5	7.5	11.0	7.40	11.9	8.1	11.6	7.50

* These values represent the sums of each calcite decomposition plus a constant, the bases found in the control soils by extraction with ammonium acetate.

carbonate would be established more positively. A Hartsells fine sandy loam and a Fullerton silt loam that were previously used as controls in the greenhouse were available for this experiment. Into samples of one hundred grams, calcite of $-200+325$ fineness was mixed at rates up to 20 m.e. per 100 g. of air-dried soil. The soils were placed in 150-ml covered beakers and kept at about 80 per cent of water holding capacity in a constant temperature room at 20°C. The moisture loss was replaced once a week. At the expiration of 90 days the soils were dried, ground to pass 0.5 mm screen, and analyzed for carbonate content. The residual carbonate, carbonate decompositions, and resultant pH values are given in Table 3. The calcite decompositions, as affected by rate of addition, are shown graphically in Figure 1. In both soils the decompositions had

reached a virtual maximum at the 12 m.e. addition. In the range between the 12 and 20 m.e. additions, the Hartsells soil shows a possible 0.3 m.e. increase in calcite decompositions, whereas the Fullerton soil shows an increased decomposition of only 0.1 m.e. According to the equilibrium

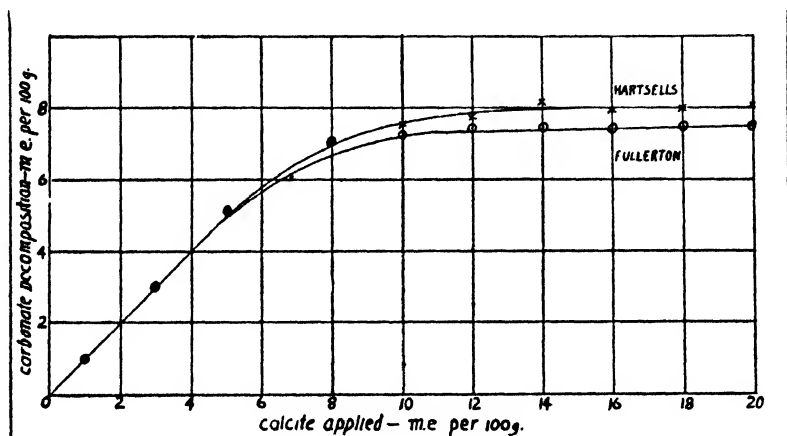


FIG. 1.—Effect of increasing additions of calcite upon extent of carbonate decomposition by Fullerton Silt Loam and Hartsells Fine Sandy Loam, after 90 days' contact.

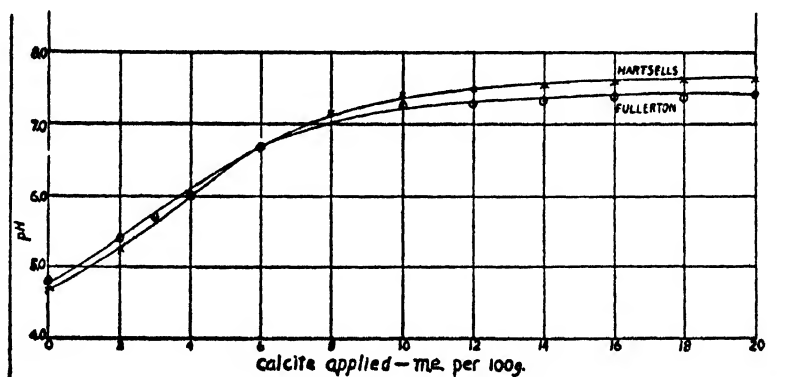


FIG. 2.—Effect of increasing additions of calcite upon pH values of Fullerton Silt Loam and Hartsells Fine Sandy Loam after 90 days' contact.

values the exchangeable hydrogen contents are 7.5 and 8.0 m.e. for the Fullerton and Hartsells soils, respectively. The curve for the pH values of the soils against the calcite additions is given in Figure 2. The maximal pH values were 7.4 and 7.5 for the Fullerton and Hartsells soils, respectively. The pH of our calcium-saturated soils again fell short of the pH 8.3 value that was indicated by the Bradfield and Allison procedure for aerated soil suspensions in the presence of an excess of calcium carbonate.

The conditions of pH measurement may account for such differences. Our soils were moistened to a loose paste, stirred and exposed overnight in a room with free interchange with outside air, and again stirred in the morning before pH measurements. The Bradfield and Allison's measurements were done usually with 10 g. of soil in 50 or 100 ml. water suspensions followed by a four-hour aeration period. When our calcium-saturated soils were leached with distilled H₂O the pH values were raised to 7.9 and 8.1 for the Fullerton and Hartsells soils, respectively. These values are within the pH range obtained by Bradfield and Allison on a Miami clay loam after a six-months' contact period with 40 m.e. of calcium carbonate (3, p. 72). There is every indication that the Fullerton and Hartsells soils were in equilibrium with the excess of calcium carbonate after 90 days' contact. The conditions necessary to effect soil-calcium carbonate equilibration in minimal contact period are now under investigation.

CARBONATE DECOMPOSITION VALUES IN RELATION TO OTHER EXPRESSIONS OF BASE-REQUIREMENT

The primary objective of the calcium carbonate-decomposition values obtained from calcium carbonate-soil equilibration was to provide a standard of comparison for any of the laboratory-derived values of exchangeable hydrogen. Of prime concern is the comparison of the barium hydroxide in barium-acetate titration values with those from carbonate decompositions. The graduated increments of calcite additions in our experiment make it possible also to obtain the exchangeable hydrogen, or lime requirement at pH 7 which is generally taken as true neutrality, or at pH 6.5 which is considered by some (2, 19) as optimal for plant growth. Because ammonium acetate is used so generally to obtain the exchange capacity and the exchangeable bases of soils, the exchangeable hydrogen is often derived from those two values: (Exch. Capacity - Exch. Bases = Exch. Hydrogen). Before a comparison is attempted it is well to consider the known limitations of each of the enumerated methods for exchangeable hydrogen determination in so far as they affect the accuracy of the expressed values.

The "carbonate decomposition" values are based on the carbonate analyses of the soil at the expiration of the experimental period in comparison with the quantity of carbonate applied or those found in the beginning of the experiment. This value can be determined with a high degree of accuracy. Strictly speaking, the carbonate decomposition value cannot be taken as identical with that of "calcium absorption" or "exchangeable hydrogen" replacement at any particular degree of saturation, because an appreciable part of the calcium reacted with the engendered biochemical end-products of sulfuric and nitric acids during the incubation period. The calcium carbonate-equivalence of those end-products may amount to as much as 1000 pounds per 2,000,000 pounds of

soil. This increment may be warranted in the designation of "exchangeable," however, on the grounds that the biochemical activities are an integral part of the soil system, and had it not been neutralized by the applied calcium carbonate, the engendered hydrogen ions would have become a part of the soil complex. When the carbonate decomposition values obtained under natural conditions are compared with any laboratory-determined titration values at equivalent states of neutralization, or *pH*, it should be borne in mind that the last-mentioned procedure will give lower values on account of the attendant experimental conditions that preclude the formation of biochemically engendered hydrogen ions.

The *pH* measurement of soil by means of the glass electrode has become the simplest routine determination in the soil-testing laboratory. On buffered solutions the glass electrode is capable of giving results of the highest precision. On as complex material as a soil, however, the *pH* is the resultant of continually shifting equilibrium between the soil particles, the water solution, and the carbon dioxide of the surrounding atmosphere. In the field, in greenhouse cultures, and in laboratory incubations, soils are affected by variable accumulations of electrolytes. Field soils are particularly affected by seasonal changes in electrolyte content (1, 9, 20, and 25). The electrolyte accumulation in field soils will cause a depression of one-half to one *pH* unit in comparison with measurements of the same soils in which the electrolytes have been removed by leaching. In practice a soil sampled after a prolonged dry and warm spell will give a considerably lower *pH* value than the same soil sampled soon after a heavy rainfall. On the other hand, *pH* readings of soils leached free of their electrolyte content are more readily affected by the carbon dioxide of the atmosphere (1, 23, 27). The significance of such *pH* fluctuations, from either of the above causes, when expressed in terms of calcium carbonate, will vary from a few hundred to several thousand pounds of calcium carbonate per acre, depending upon the buffer properties of the soil in question. The comparison of the various values for exchangeable hydrogen are given in Table 4.

CaCO₃ EQUILIBRATION VS. TITRATION TO *pH* 7 IN Ba-ACETATE

In the comparisons of Table 4, the soil-calcium carbonate equilibration results, Method A, were taken as standard, following Bradfield and Allison's definition of a calcium-saturated soil, although our results are based on actual soil-calcite contact, whereas theirs are identified with their laboratory procedure, using successively calcium hydroxide, carbon dioxide, and equilibration with carbon dioxide of the atmosphere. The titrations with barium hydroxide in .5 *M* barium-acetate, Method B, show a deviation from Method A to the extent of about 1.1 m.e. per 100 g. Since Method B was carried out under conditions which precluded the formation of biochemically engendered acids, it may be presumed in view of previous discussion, that this method accomplishes as extensive

a hydrogen replacement of the soil as does the extended contact with calcium carbonate, except for the increment resultant of nitrification and sulfonation during the incubation. This difference should not be disregarded if Method A is to be taken as standard. Furthermore, it is expected that its value would vary considerably with the type of soil and organic matter content. In order to establish a comparison of these values on a broader basis it will be necessary to extend this study on a greater number and greater variety of soils. The titration method, as

TABLE 4.—*CaCO₃ equilibration values in comparison with other methods of determination of exchangeable hydrogen of Fullerton and Hartsells soils*

METHOD OF DETERMINATION	EXCHANGEABLE HYDROGEN (m.e. PER 100 g)			
	AS DETERMINED		DERIVATIONS FROM METHOD A	
	FULLERTON	HARTSELLS	FULLERTON	HARTSELLS
A. Soil-CaCO ₃ Equilibration	7.4	8.0		
B. By titration with Ba(OH) ₂ in 0.5 M Ba-acetate-24-hrs. contact period	6.2	7.0	1.2	1.0
C. By pH vs. Ca-absorption curve (fig. 3) —unleached soils				
at pH 7.0	6.5	6.5	0.9	1.5
at pH 6.5	5.2	5.2	2.2	2.8
D. By same as C, but on leached soils				
at pH 7.0	5.3	5.4	2.1	2.6
at pH 6.5	4.0	4.2	3.4	3.8
E. By Ammonium Acetate	2.3	3.5	5.1	4.5

previously outlined (21) may well qualify as a quick procedure for determination of lime requirement. With the use of simple laboratory equipment, one person can complete 40 to 50 determinations per day.

CaCO₃ EQUILIBRATION VS. LIMING pH 7

Methods C and D are not independent methods. They represent a part of the 90 day soil-calcite incubations, similar to A in Table 4, but involve fractional calcite additions, so that varying levels of base saturation and corresponding pH values could be obtained. The pH values for C and D, in Table 4, were taken from the "Absorption Curve" of Figure 3. This curve differs from that in Figure 2, in that the pH values are plotted, not against the *calcite additions*, as in Figure 2, but against the *calcium absorptions*, as obtained from Table 3. The *titration curves*, that is, the plotting of pH values against calcium carbonate additions, are not indicative of any real relationship between the expressed variables, except where the complete decomposition of the added calcite may be presumed. This point is being emphasized because it is common to find reports on the effect of varying applications of ground limestone upon soil

pH values where the extent of calcium absorption, or carbonate decomposition, is given no consideration (2, 6, 26).

The exchangeable hydrogen indications by Method C, in Table 4, show that had the soils been limed to pH 7, and pH determined without leaching, the lime requirement would have been 6.5 m.e. for each soil. This quantity is close to that obtained by the laboratory titrations to pH 7 by barium hydroxide in .5 *M* barium-acetate. If, on the other hand,

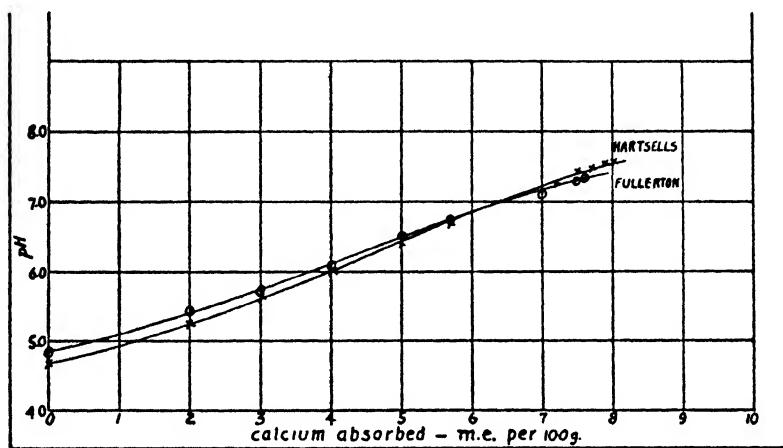


FIG. 3.—Effect of increasing calcium absorptions upon pH values of Fullerton Silt Loam and Hartsells Fine Sandy Loam.

the soil had been leached prior to pH determination, as in D, the apparent lime requirement would have been 5.3 and 5.4 m.e. for the two soils, respectively. This difference gives emphasis to the influence of electrolytes on pH value of the same soil with same degree of saturation. If 6.5 had been the desired pH, the soils should have been built up with either 5.2 m.e. or 4.1 m.e., depending upon whether the pH was determined in presence or in absence of electrolytes native to the soil after its contact with limestone. Although these facts are well known, there is no agreement among soil investigators as to how to dispose of the disturbing factor of electrolyte content of field and greenhouse soils, in making pH measurements.

The results for exchangeable hydrogen by ammonium acetate, Method E, are much lower than those obtained through the use of the other methods.

CHOICE OF LABORATORY PROCEDURE FOR EXCHANGEABLE HYDROGEN

In a search of the literature one is bewildered in finding so many related and unrelated methods for the determination of exchangeable hydrogen and lime requirement of soils. Methods range from extremes of funda-

mentals to mere changes of technique or to variance in concentration of reagents. For example, the Jones (11) calcium-acetate procedure is similar to the Brown ammonium acetate method (4) in that both are based on single contact with salts of a weak acid. The Jones method, however, prescribes use of only 1/30 of the equivalent salt concentration that is generally used with replacement methods. The serial calcium hydroxide titration method of Dunn is similar in principle to the Veitch method except for the longer contact period and the use of *pH* instead of phenolphthalein for measurement of the resultant soil reaction. The present investigation was based upon two principles: (A) that the standard for a base-saturated soil must be the actual equilibration in contact with calcite under proper temperature and moisture conditions and in contact with normal atmosphere and (B) that, for routine determination, the procedure should be simple, adapted for quantity output of easily reproducible results that would approach those obtained by calcium carbonate equilibration.

The simplified barium acetate-barium hydroxide titration procedure is deemed to meet the above-stipulated qualifications, especially since the results come nearest to the values indicated by the calcium carbonate equilibration, as shown in Table 4. Additional experimental data should be obtained concerning the relation of this titration procedure to the calcium carbonate-equilibration values. Except for the poorly buffered sandy soils, it appears improbable that the unduly high indications of liming needs would be obtained through the use of the barium acetate-barium hydroxide method, since in most instances, the laboratory titration values have been found to need "liming factors" to raise them to the higher lime requirements found in actual field trials (2, 5, 6, 19).

SUMMARY

Exchangeable hydrogen has come to be recognized as the underlying cause of soil acidity and lime requirement of soils. It is to be regretted that the *pH* determination which is being widely employed in soil-testing laboratories has only limited application for ascertaining the *quantity* of lime required to raise a soil to some desired saturation level. The exchangeable hydrogen is an indefinite quantity that varies with the method employed for its determination.

In recognizing the need of a standard for "base saturation" of soils various authorities have come to the conclusion that a "base saturated" soil is one that will not further react with an excess of calcium carbonate when exposed to ordinary atmospheric conditions. The carbonate decomposition effected by a soil in the attainment of such equilibrium is considered as equivalent to its exchangeable hydrogen content.

The present contribution deals with an attempt to supply a simple routine method for the determination of exchangeable hydrogen of soils

and to evaluate such procedure in the light of results obtained by actual contact of soils with a standard carbonate material, namely calcite. A procedure based on titration of soils with calcium hydroxide in 0.5 *M* calcium-acetate was presented in 1941 and that work has been extended to include the barium hydroxide titration in 0.5 *M* barium acetate and determinations by ammonium acetate on 13 soils. There was little difference in the values obtained by calcium hydroxide and barium hydroxide titrations, whereas ammonium acetate gave much lower results, particularly on soils of moderate to high organic matter content.

Carbonate decompositions were obtained after 90 days contact on 8 soils treated with moderate excesses of 100-mesh calcite, and on 2 soils which received treatments in greater excess and in more graduated increments. The pH values were determined and plotted against calcite additions as well as against calcium absorptions. The soils that were in equilibrium with calcium carbonate had a pH of 7.4 to 7.5, whereas the same soils leached registered a pH 8.0. At the calcium-absorption levels equivalent to the indicated base requirement by the simplified barium hydroxide-barium acetate titration, the incubated soils had a pH about 7.1. It is believed that the proposed "2-point" titration procedure for exchangeable hydrogen of soils is more in line with the base exchange principle, and gives results nearest to those obtained by soil-calcium carbonate equilibration. It is proposed that the comparison between the calcium carbonate equilibration method and the proposed titration procedure be extended to a greater number and greater variety of soil types.

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NOTE ON DETERMINATION OF METHANOL

By **GEORGE F. BEYER** (Bureau of Internal Revenue, Washington 25, D. C.)

A note on the preparation of the modified Schiff's reagent used in connection with the determination of Methanol or wherever decolorized basic Fuchsin solution is used:

It has come to the attention of the Laboratory of the Bureau of Internal Revenue that it was very difficult, if not impossible, to obtain Basic Fuchsin or Rosaniline HCl that could be decolorized with sodium sulfite and hydrochloric acid. Therefore, this Bureau made an effort to obtain some of this dye from manufacturers in the United States. It also contacted the U. S. Consulate in Switzerland in the hope of getting some of Kahlbaum's Rosaniline HCl which can be decolorized.

The samples received in the United States could not be decolorized and none was obtainable in Switzerland.

In view of this situation certain experiments were made which resulted in the following treatment. In the preparation of 250 ml of the modified Schiff's reagent, dissolve the Fuchsin or Rosaniline hydrochloric acid in about 5 ml 95% ethyl alcohol plus a little water and heat, then add the remainder of the water, sodium sulfite, and hydrochloric acid. Allow to stand at least 24 hours before using. If the solution has a brownish or amber color, add about 0.5 gram of Darco, shake well, and in about 10 minutes filter. The filtrate should be water-white.

Spectrophotometric determinations showed that the decolorized Fuchsin solution using Darco (an activated carbon) gave the same results as Kahlbaum's Rosaniline hydrochloric acid which needed no activated carbon treatment for decolorization.

BOOK REVIEWS

Les Isotopes, Rapports et Discussions, Published by the secretaries of the council under the auspices of the Scientific Commission of the Solvay Institute, Brussels. R. STROOPS, Editor—411 pages.

This book consists of a collection of papers on isotopes given at the 7th meeting of the International Solvay Institute at Brussels in 1947. There are nine reports, 7 in English and 2 in French. The authors and the subject matters discussed are as follows:

1. F. JOLIOT.—Modes de formation, constitution et filiation des isotopes artificiels.
2. K. T. BAINBRIDGE.—Some results of mass-spectrum analysis.
3. C. K. INGOLD.—Isotopes in the Spectroscopy of Polyatomic Molecules with special reference to the Benzene Molecule.
4. M. DE HEMPTINNE.—Les isotopes comme moyen d'investigation de spectres de bandes.
5. F. A. PANETH.—The preparation of radioactive Tracers.
6. A. LANGSETH.—The preparation of organic deuterium Compounds.
7. G. DE HEVESY.—Application of labeled Phosphorus.
8. M. CALVIN.—Radiocarbon and its Application in Chemistry and Biology.
9. D. RITTENBERG.—The use of N^{15} and D for the Study of chemical Processes in the living Cell.

The first 4 papers are concerned chiefly with the theoretical aspects of isotope chemistry, while the last 5 deal with practical applications. The papers by Hevesy, Calvin, and Rittenberg should prove particularly useful to the biologist and biochemist. Good bibliographies and discussions are appended to each report.

E. P. LAUG

Carl Alsberg, Scientist at Large. Edited by JOSEPH S. DAVIS. Stanford University Press, Stanford, California, 182 pages. Price \$2.00.

Older members of the Association of Official Agricultural Chemists will find this volume on the life and work of Carl L. Alsberg of especial interest. The book was planned soon after his untimely death in 1940, but the War delayed its earlier publication. Separate chapters by five of his former close associates record the growth and development of this remarkable man through the various phases of his extraordinarily productive career. The volume is edited by Joseph S. Davis, who collaborated with Alsberg at the Food Research Institute, Stanford University, and who contributes an impressive Foreword.

Readers of this Journal will doubtless find most interest in the chapter by Fred B. Linton covering Alsberg's career as Chief of the Bureau of Chemistry. This was the period during which he was active in the work of the Association of Official Agricultural Chemists and the Association of American Dairy, Food, and Drug Officials. The four remaining biographical chapters are, however, equally interesting. Their content is indicated by their titles: "The Making of the Man," "Work in the Natural Sciences," "University Professor and Administrator," and "Social Scientist Beyond the University." They demonstrate the accuracy of Dr. Davis's statement in the Foreword that "Other natural scientists and social scientists have achieved greater fame, but few have left so deep and constructive an impression on a circle so far-reaching in intellectual interests and in geographical distribution."

The book closes with three of Alsberg's own papers, which are especially well chosen, and a classified bibliography of his publications. These three papers exemplify his delightfully simple style, the broad field of his interests, and the innate modesty of this very lovable man.

P. B. DUNBAR

TUESDAY—MORNING SESSION

REPORT ON VITAMINS

By CHESTER D. TOLLE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

Vitamin A.—The recommendations of the Associate Referee are approved.

Vitamin B₁.—The recommendations of the Associate Referee are approved.

Vitamin B₂ (Riboflavin).—The recommendations of the Associate Referee on riboflavin are approved.

Vitamin C.—The recommendations of the Associate Referee are approved.

Vitamin D.—The recommendations of the Associate Referee are approved.

Nicotinic Acid.—The recommendation of the Associate Referee on nicotinic acid is approved.

Pantothenic Acid.—The Associate Referee made no report.

Folic Acid.—The extensive collaborative study conducted this year has tested the suitability of a number of methods for the determination of folic acid. Results of the study serve as a basis for selecting a single procedure for intensive study next year. The Referee recommends that during the coming year a group of well-experienced collaborators study the procedure considered by the Associate Referee to be the most suitable.

Carotene.—The recommendations of the Associate Referee on carotene are approved.

REPORT ON VITAMIN A

FURTHER COMPARISON OF THE SPECTROPHOTOMETRIC AND ANTIMONY TRICHLORIDE METHODS FOR VITAMIN A IN MARGARINE

By J. B. WILKIE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Two years ago the results of a collaborative study of the determination of vitamin A in margarine were discussed before this Association.¹ The results of this study were not entirely satisfactory, but they indicated, as more recent work has, the nature of the principal faults of the method used. It was quite clear from these results that deterioration of vitamin A occurred at certain points in the procedure. It was suggested at that time that such deterioration might be prevented by proper use of vitamin A

¹ Wilkie, J. B., Report on Vitamin A, *This Journal*, 30, 382 (1947).

stabilizers, and by increasing the speed of the chromatographing step to minimize the loss of vitamin A on the column.

Studies presented here are concerned with better use of the stabilizers normally present with vitamin A in natural sources. The use of diethyl ether in place of petroleum ether in the extraction step improves the extraction not only of vitamin A, but also of extraneous materials that may increase the stability of vitamin A. Absorption characteristics indicate that these consist chiefly of tocopherols. It is also clear from this study that the use of a smaller adsorption column is advantageous in eluting the vitamin A rapidly and with greater certainty.

The larger column previously recommended has been replaced by a smaller 1 cm diameter column, with a somewhat stronger mixture 1:1 of magnesium oxide and Celite. The solution prior to chromatographing is now evaporated partially to dryness in the presence of sodium hydrosulphite and then evaporated completely to dryness after decantation from the hydrosulphite. This more thorough evaporation eliminates extraneous volatile materials, some of which may also prove to be undesirable eluents. The material to be chromatographed is thus made more uniform from the standpoint of its behavior in the small column on which the vitamin A is more clearly defined and more sharply segregated.

With these changes in the procedure the green fluorescent band seen on the adsorption column, with the use of the weak ultraviolet lamp previously described,² is quite compact and its lower surface is sharply defined. The band can be well controlled and proceeds rapidly to the fritted exit disc of the column. The vitamin A band is then very rapidly eluted with 1 per cent of ethanol and the eluate is made to volume with petroleum ether. The density of this solution is measured at various wave lengths and the vitamin A strength is calculated from the E value obtained at 340 m μ .

A factor of 2375, which is in harmony with the 1894 U.S.P. factor, is now used to convert the $E_{1\%}^{1\text{cm}}$ values to units per gram.³

DISCUSSION

The summarized results in accompanying tables give the results obtained with this modified method. Two tables are given. Table 1 includes results from margarines labeled to contain 9,000 units of vitamin A per pound, and Table 2 from margarines labeled 15,000 units of vitamin A per pound. Values in these tables were calculated from the 325 m μ absorption, from the 340 m μ absorption, and from the antimony trichloride method as well. The tabulated coefficients of variation are es-

² Wilkie, J. B. and De Witt, J. B., *This Journal*, 28, 174 (1948), "Spectrophotometric Procedure of the E Estimation of the Vitamin A in Oleomargarine."

³ More recently the figure 1900 has been recognized as a conversion factor in connection with the adoption of vitamin A acetate as the international standard. If this figure receives general recognition the value of 2375 should be changed to 2382.

pecially revealing. As shown in the third column the actual average unitage values from the two tables calculated from the 325 $m\mu$ and the 340 $m\mu$ absorptions check within 10 per cent and 6 per cent, respectively, and the 325 $m\mu$ values are nearly always higher, as one would expect. Thus, the values based upon 340 $m\mu$ value are but little different from 325 $m\mu$ values, but, as indicated previously, they lack the extraneous absorption

TABLE 1.—*Summary of vitamin A determinations from 9000 u/lb margarines*

	U/LB				SPEC RATIO		
	Spec 325 $m\mu$ basis	Spec 340 $m\mu$ basis	Ratio		E 290 E 340	E 325 E 340	E 350 E 340
			Spec 325 Spec 340	SbCl ₃ Method			
Av. of 16 Samples	10,556	9,668	1.10	8,680	3.56	1.35	0.68
CV*	19.5	10.7	5.0	36.3	20.4	2.7	4.9

* Coefficient of variation.

TABLE 2.—*Summary of vitamin A determinations from 15,000 u/lb margarines*

	U/LB				SPEC RATIO		
	Spec 325 $m\mu$ basis	Spec 340 $m\mu$ basis	Ratio		E 290 E 340	E 325 E 340	E 350 E 340
			Spec 325 Spec 340	SbCl ₃ Method			
Av. of 68 Samples	16,122	15,645	1.06	15,360	2.7	1.32	0.678
CV*	25.5	6.9	9.86	24.0	23.0	2.4	4.8

* Coefficient of variation.

which may make the values derived from the 325 $m\mu$ values about 8 per cent high, with the possibility of greater variation at this wave length. The coefficient of variation values calculated from these data substantiate the original judgment in this respect, 10.7 and 6.9 being the CV values from the 340 $m\mu$ data, against 19.5 and 25.5 from the 325 $m\mu$ data.

The manner in which the antimony trichloride values compare with the 340 $m\mu$ values is also of interest. The antimony trichloride unitage averages were only 10 per cent and 1½ per cent lower than the 340 $m\mu$ "spec" values, but the CV values were 36.3 and 24.0, against 10.7 and 6.9 values from the 340 $m\mu$ spectrophotometric data.

The ratio of the E₂₉₀/E₃₄₀ values have a relative high CV of 23 compared

with low values of 2.4 for the E_{325}/E_{340} ratio and 4.8 for E_{350}/E_{340} ratios. This merely reflects the variability in 290 $m\mu$ region undoubtedly caused by the variable extraneous material present and emphasizes the lack of such a disturbing factor in the 325 $m\mu$ to 340 $m\mu$ region.

Thus the chromatographic spectrophotometric evaluation for vitamin A from readings at 340 $m\mu$ has greater precision than that obtained from readings at 325 $m\mu$ or from that determined by the antimony trichloride method.

It is believed that this superiority should warrant further collaborative work with the method as now modified.

PROCEDURE

(A) *Preparation of Test Solution by Saponification and Extraction.*—Weigh 10 g of oleomargarine into a 300 ml beaker and add 30 ml of boiling 95% ethanol. Stir until sample is completely disintegrated. Add 25 ml of 50% KOH soln. Stir continuously for 5 min. and allow to stand at room temp. for 15 min. (stirring occasionally).

Transfer soln to a 500 ml separatory funnel. Rinse beaker with 100 ml of distilled water in several portions, adding these rinsings to separatory funnel. Add 100 ml of U.S.P. ethyl ether. Shake vigorously, and allow to stand ca 2 minutes. Separate aqueous portion into another 500 ml separatory funnel. Likewise extract the aqueous fraction successively 4 times with 50 ml portions of ethyl ether, adding each in turn to the original extract. In case of slow separation add 2–5 ml of 95% ethanol and swirl gently.

Pour two 200-ml portions of distilled water thru combined ether extracts and discard each washing without shaking. Shake once vigorously with ca 10 ml dilute KOH soln (ca 0.02 N). Then pour distilled water with gentle agitation thru extract until it is free from alkali as shown by phenolphthalein. Allow to stand 10 min., discard the separated water, and filter with aid of vacuum thru 2.5 cm. of anhydrous Na_2SO_4 in a 2-cm fritted filter.

After adding ca 5 g of sodium hydrosulphite to the filtrate, evaporate this dry soln on steam bath in a 300-ml beaker to volume of 25–50 ml. Transfer the soln by decanting to 50 ml beaker with 6 vigorous washings of the $Na_2S_2O_4$ using 2–5 ml portions of diethyl ether to completely remove vitamin A from the residue.

Evaporate this soln to dryness on the steam bath. (Dryness is taken to mean cessation of boiling.) Heat for precisely 2 min. after the cessation of boiling, then add 5 ml of petroleum ether⁴ and make to volume in a 10 ml glass-stoppered flask with petroleum ether.

(B) *Chromatography.*—Prepare column by using small diameter tube (1 cm. in diam.) and ca 10 cm long. (Bottom of tube should be fitted with sealed-in fritted disc of porosity sufficient to offer no significant⁵ retardation to flow of solvent or eluate.)

Add 1:1 mixture of MgO (Westvaco #2641) and Celite to form column with height of 2–2.5 cm in the tube. (This material should be packed with blunt rod under 20" of vacuum to the specified height. Add 15 cm of anhydrous Na_2SO_4 on top of this column.)

Pipet 5 ml of the 10 ml test soln into a 10 ml beaker. After wetting column with petroleum ether and just before surface is dry, pour this 5-ml soln rapidly onto the

⁴ The petroleum ether when measured in a 1 cm quartz cell with an ultraviolet spectrophotometer against a no-cell blank should have a transmission of at least 85%. Better grades should have a transmission of 90%.

⁵ If the disc will allow a height of 5 ml of water to pass in less than 20 sec. the disc will probably be satisfactory in this respect.

column. A vacuum of 20" should be maintained on the column during chromatographic separation.

(The surface of the column should be kept wet with petroleum ether at all times. The segregation and progress of the vitamin A through the column is rapid and is easily followed at all times with the weak ultraviolet lamp² previously described.)

The lower surface of the A band progresses to the fritted disc with petroleum ether alone, using a 50 ml beaker in a micro-bell jar. The vacuum is then released and the eluate collected is discarded. Use a clean 50 ml beaker in the micro-bell jar to receive the eluted vitamin A. Elute this as rapidly as possible by the use of 1% of absolute ethanol in petroleum ether. The passage of the A band thru the column should be completed in less than 10 minutes.

Make the vitamin A eluate to volume of 10 ml with petroleum ether and make density determination with the spectrophotometer at 290, 325, 340, and 350 m μ , respectively. Calculate the $E_{1\%}^{1\text{cm}}$ value at 340 m μ $E_{1\%}^{1\text{cm}}$ (340 m μ) \times 2375 = U.S.P. Vitamin A units/gm margarine.

ANTIMONY TRICHLORIDE CHECK METHOD

The 5 ml of test solution remaining after removal of the spectrophotometric sample is made to 10 ml with petroleum ether. One ml of this solution is used for the antimony trichloride determination. This procedure uses distilled chloroform, 5 ml of reagent, and increment method and direct reading photometry. Since adequate details of this method have been published elsewhere, the unimportant minor modifications used in this paper will not be elaborated upon.

RECOMMENDATIONS*

After the following minor corrections are made it is recommended that the present tentative method for vitamin A in fish liver oils be made official, first action.

In sec. 36.2, page 599, the ether should be specified as "U.S.P. ethyl ether in $\frac{1}{2}$ -pound cans, anesthesia grade, free from peroxides."

In sec. 36.3, second sentence, the "ground glass joint" should be changed to "glass joint."

In sec. 36.3, seventh line, change the time from "2 minutes" to "allow the mixture to stand (about 2 minutes) until separation is visibly complete, as determined by the absence of refraction streaming and the presence of distinct layers."

Sec. 36.4, page 600, "density of 0.4" should be changed to "0.398."

Sec. 36.6, page 601, the density values given in the transmittancy-density table should be corrected to agree with the following:

T.	D.	T.	D.
0.7	2.16	29.5	0.530
7.0	1.16	32.5	0.488
11.0	0.959	55.5	0.256
26.5	0.577	63.0	0.201
27.5	0.561	71.5	0.146

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 47 (1949).

REPORT ON THIAMINE (VITAMIN B₁)

By O. L. KLINE (Division of Vitamins,* Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

In accordance with the request of the Committee on Methods the procedures for the determination of thiamine (vitamin B₁) contained in the Sixth Edition of Methods of Analysis have been reviewed in the interest of revising their status, where necessary, in preparation for the forthcoming edition of Methods of Analysis.

GROWTH METHOD

The growth method for vitamin B₁ as it appears in the Sixth Edition of Methods of Analysis was adopted as the tentative method in 1940 and was subjected to collaborative study during the following year. The method was used extensively for low potency food materials during the period of development of the chemical and microbiological assay procedures and served well as a means of checking the biological specificity of those shorter procedures. The growth method is not in wide use at the present time, but it is considered advisable to retain it for the purpose of checking biological specificity and to have it appear as an official method in the next edition of Methods of Analysis.

THE FERMENTATION METHOD

This method appears in the Sixth Edition of Methods of Analysis with status undesignated. The procedure is based upon the effect of thiamine as a stimulating agent for the rate of yeast fermentation and is finding wider use as more investigators become familiar with its easy application to a wide variety of assay materials. It has the advantage of being rapid while possessing the important characteristic of biological specificity. Yeast fermentation is stimulated by the pyrimidine of thiamine as well as by thiamine itself, necessitating the use of a sulfite blank step in which the true thiamine is inactivated in the presence of the bisulfite ion. This is of advantage in studies on stability of thiamine. In view of these advantages it is considered important therefore to retain the fermentation method for thiamine.

FLUOROMETRIC (THIOCHROME) METHOD

The thiochrome method was adopted in 1943 as tentative and following collaborative study last year (1947) was made official, first action. This method has now come to be used almost universally for the determination of thiamine in all types of materials. It has stood the test not only of extensive collaborative study but of wide use in many countries.

In the interest of widening the application of the official method as it

* E. M. Nelson, Chief.

appears in the Sixth Edition of Methods of Analysis certain minor definitive changes are needed.

RECOMMENDATIONS*

It is recommended—

(1) That the growth method for thiamine (vitamin B₁) be adopted as official, first action.

(2) That the fermentation method for the determination of thiamine be made official, first action.

(3) That sec. 36.25 (Preparation of assay solution) be changed as recommended in the report of the Associate Referee.†

REPORT ON RIBOFLAVIN (MICROBIOLOGICAL)

By HENRY W. LOY, Jr. (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

The tentative microbiological method for the determination of riboflavin (*This Journal*, 30, 79) was studied collaboratively (*This Journal*, 24, 413; 25, 459; 26, 81; 28, 560; 29, 25; and 31, 701). This method has been in use since such adoption and is regarded as suitable. Furthermore, this year's work on the chemical method for the determination of riboflavin included a comparison with the microbiological method. The results are included in that report (*This Journal*, page 461). More uniform results have been obtained in the later studies of the microbiological method than were obtained in the earlier studies, indicating that experience with the method is helpful in its proper application.

RECOMMENDATION‡

It is recommended that the tentative microbiological method for the determination of riboflavin, as revised, be made official, first action.

REPORT ON RIBOFLAVIN (CHEMICAL)

By HENRY W. LOY, Jr. (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

Last year, it was recommended that further work be conducted on the fluorometric method (*This Journal*, 30, 392; 31, 701) that was the subject of that year's study. Although many of the collaborators for last year obtained results that were in good agreement with those obtained by the microbiological method, there was a wide divergence of some of the

* For report of Subcommittee A and action by the Association, see *This Journal*, 32, 47 (1949).

† Details of the revision are given in *This Journal*, 32, 104 (1949).

‡ For report of Subcommittee A and action of the Association, see *This Journal*, 32, 48 (1949). Details of the revised method are given in *This Journal*, 32, 105 (1949).

results. Therefore, the study did not lead to the recommendation for adoption of a fluorometric method, but it seemed desirable to determine if further experience with the method would lead to more uniform results.

The fluorometric method used in this year's study was the same as the one used in last year's study, except for a few minor modifications, related to more specific directions for some steps of the procedure. The collaborators were asked to submit results obtained by the specified fluorometric method, and, where possible, by the tentative microbiological method (*This Journal*, 30, 79) and any other methods in which they might be interested, for 3 samples: No. 1, a solution of riboflavin¹; No. 2, an enriched flour; and No. 3, a dried brewers' yeast.

Of the 9 collaborators who submitted results by the specified fluorometric method, 5 submitted results by the tentative microbiological method. These collaborators also submitted results in 4 cases by fluorometric methods that differed from the specified method, and in 1 case, by a microbiological method that differed from the tentative method.

TABLE 1.—Results of collaborative study on Riboflavin

COLLABORATOR NO.	FLUOROMETRIC METHODS		MICROBIOLOGICAL METHODS	
	COLLABORATIVE	OTHER	TENTATIVE	OTHER
Sample No. 1—Riboflavin Solution (Mg. of riboflavin per ml. of sample)				
1	0.401			
2	0.39	0.39		
3	0.399		0.400	
4	0.40	0.40	0.40	
5	0.383		0.438	
6	0.381	0.373	0.396	
7	0.40			
8	0.390			
9	0.400		0.400	0.420
Av. \pm S.E. of Av. for the 9 collaborators on the fluorometric method	0.394 ± 0.003			
Av. \pm S.E. of Av. for the 5 collaborators on both methods	0.393 ± 0.004		0.407 ± 0.008	
Student's <i>t</i> value for comparing both methods			-1.56	
P value			-0.16	
Comment	No significant difference			

¹ This sample was a 0.02 *N* acetic acid solution containing, per ml, 0.4 mg of U.S.P. Riboflavin Reference Standard, 100 mg of urea, and 5.0 mg of chlorobutanol.

TABLE 1—(continued)

COLLABORATOR NO.	FLUOROMETRIC METHODS		MICROBIOLOGICAL METHODS	
	COLLABORATIVE	OTHER	TENTATIVE	OTHER
Sample No. 2—Enriched Flour (Mg. of riboflavin per lb. of sample)				
1	1.00			
2	0.96	1.15		
3	0.98		0.98	
4	1.03	1.01	1.09	
5	1.09		1.04	
6	1.01	1.00	1.08	
7	1.04			
8	1.10			
9	1.05		1.12	1.03
Av. \pm S.E. of Av. for the 9 collaborators on the fluorometric method	1.03 ± 0.02			
Av. \pm S.E. of Av. for the 5 collaborators on both methods	1.03 ± 0.02		1.06 ± 0.02	
Student's <i>t</i> value for comparing both methods			= 1.06	
P value			= 0.32	
Comment	No significant difference			
Sample No. 3—Dried Brewers' Yeast (Mg. of riboflavin per g. of sample)				
1	0.0399			
2	0.04	0.04		
3	0.0396		0.0397	
4	0.041	0.042	0.041	
5	0.041		0.041	
6	0.0431	0.0425	0.0390	
7	0.041	0.043		
8	0.0416			
9	0.0525		0.0413	0.0435
Av. \pm S.E. of Av. for the 9 collaborators on the fluorometric method	0.0422 ± 0.0013			
Av. \pm S.E. of Av. for the 5 collaborators on both methods	0.0434 ± 0.0023		0.0404 ± 0.0004	
Student's <i>t</i> value for comparing both methods			= 1.28	
P value			= 0.24	
Comment	No significant difference			

The results are shown in Table 1. In only one case, collaborator 9, sample No. 3, in the specified fluorometric method, was there a wide divergence in the results. There is no explanation to offer for this wide divergence. Except for this one case, the results obtained by the specified fluorometric method are more uniform than those of last year's study and compare favorably with results obtained by the tentative microbiological method. From the results of the 5 collaborators who used both methods, the average results by the specified fluorometric method and the tentative microbiological method are, respectively, 0.393 and 0.407 mg. per ml., for sample No. 1; 1.03 and 1.06 mg. per lb., for sample No. 2; and 0.0434 and 0.0404 mg. per g., for sample No. 3. As can be seen from Table 1, in no instance is there a statistically significant difference between the results of the two methods.

This study indicates that experience with the fluorometric method is helpful in its proper application. This has been found true with the microbiological method as well (*This Journal*, 32, 105).

COLLABORATORS

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 Elmer De Ritter, Hoffmann-La Roche, Inc., Nutley, N. J.
 Norman E. Foster, Food and Drug Administration, Philadelphia, Pa.
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 F. G. McDonald, Mead Johnson and Company, Evansville, Ind.
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 Victor B. Williams and Laura M. Flynn, Univ. of Missouri, Columbia, Mo.

The Associate Referee wishes to express appreciation to the collaborators and to their organizations for their cooperation in this study.

RECOMMENDATION*

It is recommended that the fluorometric method† for the assay of riboflavin herein described be made official, first action.

REPORT ON FOLIC ACID

By LAURA M. FLYNN (Dept. of Agricultural Chemistry, University of Missouri, Columbia, Mo.), *Associate Referee*

The microbiological method for determining folic acid was subjected to collaborative assay under the auspices of the Association of Official Agricultural Chemists in 1947. Results showed fair agreement among the seventeen laboratories participating in the study. However, the findings in this first collaborative investigation of the vitamin (1) indicated the need for further study of its determination.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 47 (1949).

† The details of the method are given in *This Journal*, 32, 108 (1949).

Both *Lactobacillus casei* and *Streptococcus faecalis* have been used as test organisms in the microbiological assay of folic acid. *S. faecalis* grows well in a semi-synthetic medium, is less dependent on unidentified growth stimulants and is often read turbidimetrically after 18 hours' incubation. It does not respond to folic acid conjugates. On the other hand, *L. casei* shows greater acid production, and is sensitive to the vitamin over a wider range and at a lower concentration. When turbidity is used as the criterion of growth dose-response curves for standards and for unknowns do not show parallelism until a long incubation period (40 hours or more) has elapsed, when *L. casei* is used. Comments on the 1947 assays indicated that peptone is not a satisfactory source of the unidentified growth substances known to be necessary when this organism is used in folic acid assays. *L. casei* was selected for the 1947 study, and since half of those replying to the questionnaire sent with the 1947 samples indicated a preference for this bacterium, collaborators were asked to try it again in the 1948 investigation.

Roberts and Snell (2) have recently proposed a medium for *L. casei* which might improve materially the assay for pteroylglutamic acid. Its content of trypsinized casein should insure rapid early growth of the organism, thus overcoming one of the main objections to the use of *L. casei*. For small laboratories, however, which often do occasional assays for several vitamins, it seems advantageous to use, whenever possible, only materials which are commercially available, constant in composition, and easily stocked and stored in quantity. This would cut to a minimum the time used by technicians in digestions, adsorptions, and the preparation of special supplements of limited use. The utilization of materials commercially available should also make possible greater uniformity between laboratories in a collaborative assay.

Several years ago Davis and Dubos (3) demonstrated that the early growth of tubercle bacilli in submerged culture in liquid media was facilitated by the addition of the commercial product "Tween 80" (polyoxyethylene sorbitan monooleate). Unpublished data compiled in the vitamin assay laboratory of the Department of Agricultural Chemistry at the University of Missouri have proved that "Tween 80" markedly stimulates the early growth of *L. casei* also, in a semi-synthetic medium. Further studies in this laboratory showed that a modified Landy and Dicken medium containing "Tween 80" provides a very acceptable medium for use in folic acid assays. Since this medium had been used successfully for this purpose over a period of several months in the laboratory of the Associate Referee it was selected as the test medium for the 1948 study of the vitamin. This medium differs from the Teply and Elvehjem medium (4) which was used last year, as follows: Substitution of cysteine for cystine, omission of peptone and alanine, minor changes in the vitamin mixture, increase in the amount of manganous sulfate used, and the

addition of "Tween" and glutathione. The medium is easily prepared, is reproducible, and by altering the buffer it can be used successfully with either *L. casei* or *S. faecalis*. It gives much better results with *L. casei* than the original Landy and Dicken medium (5).

Detailed directions for the method were sent to all laboratories which had expressed willingness to participate. Chemists co-operating in the study were asked to assay the samples by any other methods they were using routinely, if time permitted. Five laboratories accustomed to making chick assays for folic acid agreed to assay the samples by chick methods.

The materials chosen for assay in 1948 were (1) dehydrated powdered mustard greens, (2) soy flour, (3) dehydrated powdered egg yolk, and (4) brewers' yeast. In order to insure as much uniformity as possible among the laboratories each collaborator was sent a desiccated culture of bacteria, crystalline pteroylglutamic acid for use as a standard, and sufficient desiccated chicken pancreas for the enzyme hydrolysis of the samples. It was requested that the culture be stored under refrigeration until used, then sub-cultured several times in a complete medium containing liver extract before its utilization as inocula or storage on agar.

At the present time no material is available which is recognized as a reference standard for folic acid, although steps looking toward the development of a reliable reference standard have been taken by the proper authorities. In the absence of an official standard the synthetic pteroylglutamic acid to be sent to collaborators (Folvite 7-7904) was checked in the laboratory of the Associate Referee. A Beckman spectrophotometer was used in the measurement of extinctions at wave lengths 255, 282.5, and 365 millimicrons. $E_{1\text{cm}}^{1\%}$ values at these wave lengths were, respectively, 555, 545, and 190. The vitamin was tested at pH 11.5, at a concentration of 0.002%. The data given here will serve to characterize the material used as a standard by collaborators. Physical constants for the compound, including extinction data, have been published by Parke, Davis and Company (6) and by the Lederle Laboratories Division of the American Cyanamid Company (7). From extinction data for crystalline folic acid, as published by Parke, Davis and Co., chemists, the material used by collaborators would be calculated to be about 90 per cent pure. On the basis of the extinction data for the crystalline compound as published by chemists at the Lederle Laboratories, the same material would be considered 98 per cent pure.

COLLABORATORS

The chemists co-operating in this study are listed below. Grateful acknowledgement is made of the generous and gracious help of all who took part in the endeavor.

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RESULTS

Assay results submitted by the co-operating laboratories are summarized in Tables 1-6. To facilitate comparisons, arithmetic means and median values are listed in the tables. In the calculation of these means and medians only one figure was used for each laboratory, an average of the several figures from each laboratory as shown in the tables. When a laboratory submitted several assay results the range of these is indicated in the tables. Frequently laboratories sent in only one estimate for each sample, but this estimate represented the average from several assays on the material. Several of the estimates listed, as is shown in the footnotes, were not included in the averages. In one case (Laboratory 12) the chemist sending in the data specified which one of his tests he considered best, giving the reasons, and only that figure was included. The Associate

* Assayed samples by both microbiological and chick methods.

TABLE 1.—Results of 1948 A.O.A.C. collaborative study of folic acid
(Assays with *Lactobacillus casei* in specified medium)

LAB. NO.	METHOD OF EVALUATION	SAMPLE I MUSTARD GREENS		SAMPLE II SOY FLOUR		SAMPLE III EGG YOLK		SAMPLE IV BREWERS' YEAST	
		AVE.*	RANGE	AVE.*	RANGE	AVE.*	RANGE	AVE.*	RANGE
(1)	Acidimetric	8.76 (4)	4.98-16.16	3.49 (3)	2.62-4.76	0.828 (3)	0.60-1.00	16.10 (2)	14.50-17.70
(2)	Acidimetric	6.57 (1)		2.14 (1)		0.093 (1)†		18.1 (1)	
(3)	Acidimetric	10.38 (1)		4.12 (2)	3.96-4.29				
(4)	Turbidimetric	8.95 (2)	8.86-9.03	4.27 (2)	4.02-4.27				
(5)	Turbidimetric	9.3 (1)		3.7 (1)					
(6)	Acidimetric	Free		Free		0.82 (1)		19.7 (1)	
(7)	Acidimetric	3.0 (1)		1.1 (1)		Free			
(8)	Acidimetric	Total		1.1 (1)		0.37 (1)			
(9)	Acidimetric	9.4 (1)		4.8 (1)		Total		Total	
(10)	Turbidimetric	7.32 (3)		5.34 (3)		0.48 (1)		21.7 (1)	
(11)	Turbidimetric	6.7 (1)		4.0 (1)		0.966 (3)		16.01 (3)	
(12)	Acidimetric	9.9 (3)	9.3-10.3	5.8 (3)	5.6-6.1	1.2 (1)		18.0 (1)	
(13)	Acidimetric	6.87 (3)†	6.5-7.4	3.27 (3)†	3.0-3.6	1.9 (1)		24.5 (3)	22.6-27.0
(14)	Acidimetric	7.4		3.6		0.84 (3)†	0.65-1.00	17.3 (3)†	14.4-19.9
(15)	Acidimetric	9.29 (4)	8.27-9.94	3.48 (4)	3.30-3.75	1.0		19.9	
(16)	Acidimetric	8.41 (1)†		3.11†		0.722 (4)	0.471-0.963	16.18 (4)	15.29-16.80
Mean	Acidimetric	8.71 (4)	6.7-9.9	4.25 (5)	3.49-5.8	0.65†		13.2†	
Median	Acidimetric	8.45 (8)	6.57-10.38	3.91 (6)	2.14-5.34	1.19 (4)	0.82-1.90	19.33 (4)	16.10-24.50
	Turbidimetric	8.95-9.3 (4)	6.7-9.9	4.00 (5)	3.49-5.8	0.72 (4)	0.48-1.00	18.18 (5)	15.01-21.70
	Acidimetric	7.50-8.76 (8)	6.57-10.38	3.60-4.12 (6)	2.14-5.34	0.83-1.20 (4)	0.82-1.90	18.00-19.7 (4)	16.10-24.50
Over-all Mean	Turbidimetric & Acidimetric	8.54 (12)	6.57-10.38	4.07 (11)	2.14-5.8	0.87-0.72 (4)	0.48-1.00	18.10 (5)	15.01-21.70
Over-all Median	Turbidimetric & Acidimetric	8.76-8.95 (12)	6.57-10.38	4.0 (11)	2.14-5.8	0.92 (8)	0.49-1.90	18.66 (9)	15.01-24.50
						0.82-0.83 (8)	0.49-1.90	18.10 (9)	15.01-24.50

* Numbers in parentheses indicate number of determinations included in average.

† Not included in calculating mean and median.

Referee did not include the data from Laboratory 15, using *L. casei*, in the averages because the blank was excessively high. To show the range of results in the hands of many technicians (of varying degrees of experience in microbiological assays of folic acid) it seems worthwhile, however, to present in the tables all the data submitted.

The fifteen laboratories submitting data from microbiological assays sent to the Associate Referee detailed information on at least two hundred microbiological determinations on the four samples tested. Assayists will appreciate the enormous amount of work involved in so large a number of microbiological tests, as well as the effort expended in the time- and labor-consuming chick assays made in five laboratories.

A comparison of results obtained when a laboratory carried out a microbiological assay by more than one method, or with more than one organism on the same day, demonstrates that the varied procedures gave (within experimental error) almost identical results. Occasionally this was not true.

Results compiled in Table 1 (from assays with *L. casei* in the medium specified for the assay) indicate remarkably good agreement between laboratories. This is particularly encouraging since the data on "free folic acid," for which we are indebted to Laboratory 8, prove that the larger portion of the total folic acid in each sample (with the possible exception of the egg yolk) is in the form of conjugates. It is very satisfying, also, to find that results from turbidimetric assays check closely with the results from assays evaluated by titration.

When *S. faecalis* was used as the test organism in the specified medium, there was again good agreement among the results from various laboratories and between acidimetric and turbidimetric determinations. Although fewer data were reported with *S. faecalis* than with *L. casei* in the medium specified for the collaborative test, results shown in Table 2 prove that estimates from assays with the two organisms were in harmony with one another.

Four laboratories contributed data showing comparison of results from assays with *L. casei* in the specified medium and in other commonly used media. These data are shown in Table 3. Determinations made in Laboratory 3 showed that assays with *L. casei* in the Roberts and Snell medium yielded results which paralleled those obtained with *L. casei* or *S. faecalis* in the medium used for the collaborative study. Data showing results from assays with *L. casei* in the Teply and Elvehjem medium are too few to justify comparison with results in the other media. In Laboratories 9 and 12 results from the several methods were in excellent agreement. In Laboratory 13 results from the Teply and Elvehjem medium were slightly lower than those from the A.O.A.C. medium. In general it is observed that assay results from assays with *L. casei* in the various media are in good agreement.

TABLE 2.—Results of 1948 A.O.A.C. collaborative study on folic acid
(Assays with *Streptococcus faecalis* in specified medium)

LAB. NO.	METHOD OF EVALUATION	SAMPLE I MUSTARD GREENS		SAMPLE II SOY FLOUR		SAMPLE III EGG YOLK		SAMPLE IV BREWERS' YEAST	
		Ave.*	RANGE	Ave.*	RANGE	Ave.*	RANGE	Ave.*	RANGE
2	Turbidimetric	5.13 (2)	10.62-11.67	2.63 (1)	5.0-5.61	0.192 (1)	0.5-1.29	11.47 (1)	
3	Acidimetric	9.8 (1)		4.2 (1)		0.65 (1)		22.0 (1)	
3	Turbidimetric	11.10 (2)		5.30 (2)		0.895 (2)		22.54 (1)	
7	Acidimetric	8.14 (1)		4.07 (1)		1.26 (1)		19.60 (1)	
8	Acidimetric	Free		Free		Free		Free	
		1.47 (1)		0.50 (1)		0.44 (1)		0.54 (7) (1)	
		Total		Total		Total		Total	
9	Acidimetric	10.70 (1)		5.5 (1)		0.65 (1)		24.4 (1)	
13	Turbidimetric	7.38 (1)		3.33 (1)		1.08 (1)		17.09 (1)	
		9.38 (1)		5.03 (1)		0.526 (1)		24.15 (1)	
Mean	Turbidimetric	8.54 (3)		4.32 (3)		1.11 (3)		19.39 (3)	
	Acidimetric	8.76 (4)		4.27 (4)		0.91 (4)		20.77 (4)	
Median	Turbidimetric	9.38 (3)		5.03 (3)		0.895 (3)		22.54 (3)	
	Acidimetric	8.14-9.80 (4)		4.07-4.20		0.65-1.08 (4)		19.6-22.0 (4)	
Overall Mean	Turbidimetric & Acidimetric	8.77 (7)		4.29 (7)		0.997 (7)		20.15 (7)	
Overall Median	Turbidimetric & Acidimetric	9.38 (7)		4.20 (7)		0.895 (7)		22.0 (7)	

* Numbers in parentheses indicate number of results included in average.

TABLE 3.—Results of 1948 A.O.A.C. collaborative study on folic acid
(Assays with *L. casei* in media other than that specified)

LAB. NO.	METHOD OF EVALUATION	MEDIUM	SAMPLE I MUSTARD GREENS		SAMPLE II SOY FLOUR		SAMPLE III EGG YOLK		SAMPLE IV BREWERS' YEAST	
			AVE.*	RANGE	AVE.*	RANGE	AVE.*	RANGE	AVE.*	RANGE
3	Turbidimetric	Roberts and Snell	mmg/g	mmg/g	mmg/g	mmg/g	mmg/g	mmg/g	mmg/g	mmg/g
	Acidimetric	Roberts and Snell	10.93 (1)	5.095 (1)	0.581 (1)	0.581 (1)	30.243 (1)	30.243 (1)	28.56 (1)	28.56 (1)
9	Acidimetric	Modified Teply and Elvehjem	10.00 (1)	5.00 (1)	0.556 (1)	0.556 (1)	—	—	—	—
12	Acidimetric	Teply and Elvehjem	6.94 (1)	5.09 (1)	0.65 (1)	0.65 (1)	17.7 (1)	17.7 (1)	—	—
13	Acidimetric (†)	Teply and Elvehjem†	6.5 (1)	3.2 (1)	0.49 (1)	0.49 (1)	15.36 (1)	15.36 (1)	—	—
		Teply and Elvehjem‡	7.5 (1)	3.32 (1)	0.697 (4)	0.697 (4)	15.62 (4)	15.62 (4)	14.12-17.09	14.12-17.09
			7.96 (4)	3.30 (4)	3 10-3 67	3 10-3 67	0.539-0.870	0.539-0.870	—	—
Over-all Mean			8.31 (6)	4 17 (6)	0.595 (5)	0.595 (5)	0.490-0.697	0.490-0.697	21.45 (5)	15.36-30.24
Over-all Median			7.50-7.96 (6)	3.32 -5 00 (6)	0.581 (5)	0.581 (5)	0.490-0.697	0.490-0.697	17.70 (5)	15.36-30.24

* Numbers in parentheses indicate number of results included in average.

† Hydrolyzed with hog kidney enzyme.

‡ Hydrolyzed with chicken pancreas enzyme.

TABLE 4.—Results of 1948 A.O.A.C. collaborative study on folic acid
(Assays with *S. faecalis* in media other than that specified)

LAB. NO.	METHOD OF EVALUATION	MEDIUM	SAMPLE I MUSTARD GREENS		SAMPLE II SOY FLOUR		SAMPLE III EGG YOLK		SAMPLE IV BREWERS' YEAST	
			AVE.*	RANGE	AVE.*	RANGE	AVE.*	RANGE	AVE.*	RANGE
2	Turbidimetric	—	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g
4	†	?	29.17†	16.82†	16.82†	9.07†	9.07†	31.04†		
5	Turbidimetric	Modified Teply and Elvehjem	4.8 (1)	3.4 (1)	3.4 (1)	0.55 (1)	0.55 (1)			
6	?	Rabinowitz and Shell	7.3 (1)†‡	3.8 (1)†‡	3.8 (1)†‡	0.66 (1)†‡	0.66 (1)†‡	19.6 (1)†‡		
8	Turbidimetric	?	6.8 (1)	3.6 (1)	3.6 (1)	0.66 (1)	0.66 (1)	13.3 (1)		
11	Turbidimetric	Teply and Elvehjem	10.5 (1)	6.35 (1)	6.35 (1)	—	—	21.6 (1)		
14	?	Teply and Elvehjem	12.4 (1)	7.1 (1)	7.1 (1)	3.9 (1)	3.9 (1)	30.2 (1)		
			12.35 (2)	5.4 (2)	5.4 (2)	0.73 (2)	0.73 (2)	22.15 (2)		
Over-all Mean			9.21 (5)	4.80-12.40	5.17 (5)	3.40-7.10	1.46 (4)	0.55-3.90	21.81 (4)	12.30-30.2
Over-all Median			10.50 (5)	4.80-12.40	5.40 (5)	3.40-7.10	0.66-0.73 (4)	0.55-3.90	21.60-22.15 (4)	13.80-30.2

* Numbers in parentheses indicate the number of results included in average.

† Not included in calculation of mean and median.

‡ A different pteroylglutamic acid standard was used here.

The estimates of folic acid based on assays with *S. faecalis* in media other than that specified for collaboration are summarized in Table 4. These data are too few to warrant more than brief comment. Checking these results with other results from the same laboratories, however, reveals good agreement within each laboratory in tests with *S. faecalis*, regardless of the medium chosen.

Results from chick assays in several laboratories have been assembled in Table 5. The values listed for Laboratory A were averaged in the office of the Associate Referee from data submitted by the aforementioned laboratory, showing estimates at several dosage levels for the test materials. The range of the estimates averaged is indicated. The values listed for Laboratory B were calculated by these assayists as tentative estimates, pending a statistical evaluation of their data by the Method of Least Squares. The data shown in Table 5 indicate good agreement in the reports from the different laboratories. As pointed out by the chemist submitting the report for Laboratory C, "assays based on total cell count and hematocrit are in agreement with the microbiological results but are divergent from the weight assay data. The reasons for this are not known but may be due to an influence of the 'liver principle.'" The divergence between folic acid estimates based on chick weight and those based on blood tests or microbiological tests is particularly marked in the case of the dried egg yolk. This material may be a comparatively poor source of folic acid, but it apparently is an excellent source of unidentified growth stimulants for both chicks and bacteria.

Data showing averages from assays by various methods are compiled in Table 6, to enable comparison of results from the different methods. This summary reveals excellent agreement among folic acid estimates from assays by varied microbiological procedures. From these findings one may conclude that it is possible to obtain very good results from assays with either *L. casei* or *S. faecalis*, using the organisms in any one of several media.

INFORMATION FROM QUESTIONNAIRES

Standard cultures.—Collaborators were asked to submit specific data from acidimetric or turbidimetric tests, showing bacterial growth in standard and test cultures in folic acid assays. Almost without exception these data were excellent. When *L. casei* was used in the medium specified the blanks were low (usually one ml. or less of 0.1 *N* acid) and the acid production was high (10 to 14 ml. of 0.1 *N* acid at a dosage of one millimicron of folic acid). Several collaborators compared *L. casei* in the medium suggested by the Associate Referee with *L. casei* in the Teply and Elvehjem medium, and felt that the medium with "Tween" and glutathione gave lower blanks, better acid production, and more satisfactory results in general. It was pointed out last year by several collaborators that *L.*

TABLE 5.—Results of 1948 A.O.A.C. collaborative study on folic acid
(Assays by chick methods)

LAB. NO.	METHOD OF EVALUATION	SAMPLE I MUSTARD GREENS		SAMPLE II SOY FLOUR		SAMPLE III EGG YOLKS		SAMPLE V SPINACH	
		AVE.	RANGE	AVE.	RANGE	AVE.	RANGE	AVE.	RANGE
A	Weight	mmg/g 13.3	mmg/g 12.0-15.5	mmg/g 2.9	mmg/g 2.0-5.5	mmg/g 4.3	mmg/g —	mmg/g 15.3	mmg/g 14.6-16.1
B	Weight	14.4		5.5		2.8		18.6	
	Hematocrit	13.6		4.6		1.4		16.9	
	Average	14.0		5.0		2.1		17.8	
C	Chick Weight	14.6							
	Chick Thymus	14.7							
	Weight								
	Total Cell	8.6							
	Count	10.0							
	Hematocrit	12.0							
	Average								
D	Weight	—		1.8*				3.3*	
	Hemoglobin	7.0*		2.6*		1.7*		5.3*	
E	Weight	13†		4.42		1.43		14.8	

* Ration contained supplement of anti-pernicious anemia liver extract, to supply "animal protein factor."
† 13 assumed to be correct value for mustard greens.

TABLE 6.—Results of 1948 A.O.A.C. collaborative study on folic acid
(Comparison of results of assays by several methods)

METHOD OF ASSAY	SAMPLE I		SAMPLE II		SAMPLE III		SAMPLE IV	
	AVE.*	RANGE	AVE.*	RANGE	AVE.*	RANGE	AVE.*	RANGE
1. <i>L. casei</i> in medium specified	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g
Mean	8.54 (12)	6.57-10.38	4.07 (11)	2.14-5.8	0.932 (8)	0.48-1.90	18.69 (9)	15.01-24.50
Median	8.76-8.95 (12)	6.57-10.38	4.0 (11)	2.14-5.8	0.82-0.83 (8)	0.48-1.90	18.10 (9)	15.01-24.50
2. <i>S. faecalis</i> in medium specified	Mean	8.77 (7)	5.13-11.10	4.29 (7)	2.63-5.50	0.997 (7)	20.18 (7)	11.47-24.4
	Median	9.38 (7)	5.13-11.10	4.20 (7)	2.63-5.50	0.895 (7)	22.0 (7)	11.47-24.4
3. <i>L. casei</i> in other media	Mean	8.31 (6)	6.50-10.93	4.17 (6)	3.20-5.095	0.595 (5)	21.48 (5)	15.36-30.24
	Median	7.5-7.96 (6)	6.5-10.93	3.32-5.00 (6)	3.20-5.095	0.581 (5)	17.70 (5)	15.36-30.24
4. <i>S. faecalis</i> in other media	Mean	9.21 (5)	4.8-12.4	5.17 (5)	3.4-7.1	1.46 (4)	21.81 (4)	13.3-30.2
	Median	10.5 (5)	4.8-12.4	5.4 (5)	3.4-7.1	0.66 (4)	21.6-22.15 (4)	13.3-30.2
5. Chick tests								
	Laboratory A	13.3	12.0-15.5	2.9	2.0-5.5	4.3		
	Laboratory B	14	13.6-14.4	5.0	4.6-5.5	2.1		
	Laboratory C	12	8.6-14.7					
	Laboratory D†	7.0		2.6		1.7		
Laboratory E‡	13		4.42		1.43			

* Numbers in parentheses indicate number of results averaged.

† Bacon contained supplement of anti-pernicious anemia liver extract, to supply "animal protein factor."

‡ 13 assumed to be correct value for mustard greens

casei gives very acceptable results in a modified Teply and Elvehjem medium, without peptone, but using commercial enzyme-hydrolyzed casein as the source of both amino-acids and growth stimulants. *S. faecalis* also gave very acceptable results in the medium suggested by the Associate Referee, or in a modified Teply and Elvehjem medium.

Basal media.—Very few comments concerning media were made by collaborators. One suggested that the stock solutions specified for the collaborative medium were too dilute for easy handling; he preferred them about four times as strong. Another felt that the medium suggested was more complex than was justified by its advantages; he likes as a medium for *L. casei* the old Mitchell-Snell medium with pyridoxine increased and using Difco Casamino acids as a source of hydrolyzed casein (8). Four remarked that they liked the medium suggested by the Associate Referee for *L. casei*.

Choice of test organism.—Of the twelve collaborators expressing a preference eight prefer *S. faecalis* for folic acid assays, while four liked *L. casei* better. It is agreed that it is easier to get low blanks with *S. faecalis*, that it can be read turbidimetrically after a very short incubation period, and that its nutritive requirements are less fastidious. *L. casei*, however, gives markedly better acid production, shows greater sensitivity to the vitamin, and is sensitive to it at a lower level of potency. The following comments by three collaborators are typical of the opinions of others: Laboratory 5, "*S. faecalis* when used with the medium of Teply and Elvehjem but omitting peptone supplement gives more consistent and reproducible results. Also it is much easier to obtain proper blanks with *S. faecalis*;" Laboratory 8, "We like *S. faecalis*—we have had much greater experience with it and it is faster. Obviously on some determinations *L. casei* must be used." Laboratory 13, "We prefer *L. casei*. In our experience it has given more consistent results on day-to-day testing and in replicate cultures on the same day."

From the data submitted by many collaborators it seems obvious to the Associate Referee that either organism may be used with excellent results for folic acid tests.

Purification of casein hydrolysates.—Whenever it is wished to use *L. casei* in assays of folic acid it is essential that the hydrolyzed casein used in the medium shall be very low in the vitamin. From data submitted in this collaborative assay it is evident that *S. faecalis* responds quantitatively to the vitamin in the range 0.5 to 5 (or perhaps to 6 or 7) millimicrograms per 10 ml. of culture. *L. casei* is sensitive to the vitamin in the range 0.1 to 1 (or probably 2) millimicrograms per 10 ml. culture. Several of the chemists co-operating in this study commented on special adsorptions needed to make casein hydrolysates suitable for use with *L. casei* in folic acid tests. Laboratory 1 found it necessary to treat the hydrolysate

with Norite after an earlier treatment with Nuchar; Laboratory 6 observed that they obtained lower blanks with GBI vitamin-free casein after treating it with Norite; Laboratory 7 had difficulty with high blanks in tests with *L. casei*, and subsequent tests on various casein hydrolysates made it seem very possible that their earlier difficulties were caused by folic acid in the casein. Laboratory 3 finds that Darco is a suitable adsorbent for freeing Difco casamino acids from folic acid, for use in tests for the vitamin.

Extraction procedures.—Only one laboratory submitted data showing a comparison of findings when a variation was made in the extraction procedure. Data submitted by Laboratory 13 (listed in Table 3) show very little difference in results when hog kidney enzyme was substituted for chicken pancreas enzyme in the hydrolysis of samples. It is apparent that the suggested procedure for extraction is adequate, in the assay of the mustard greens, soy flour, brewers' yeast, and of the spinach sample assayed collaboratively last year. Very possibly the procedure for extraction of the egg yolk needs further study.

In the 1947 collaborative study of folic acid, reports on the microbiological assay of the spinach sample showed a mean value of 12.89 mmg./g., and median values of 12.9–13.7 mmg./g. using *L. casei* in the Teply and Elvehjem medium. Three laboratories, all experienced in folic acid assays, reported values of 14.4 to 17 mmg./g. Two laboratories reported results from chick assays, 11 and 16 mmg./g. Because of the divergence of these findings further study was made of the potency of the sample. The desiccated spinach was stored at -40° and was submitted to chick assay again in 1948. Data on the spinach as listed in Table 5 are in harmony with the higher results from microbiological assays in the 1947 study.

In commenting on extraction procedures, attention should be called to data submitted by Laboratory 2, as shown in Table 4. The results listed were obtained in assays after the samples were submitted first to treatment with papain (Caroid) and takadiastase (Mylase P) before the addition of pancreas enzymes. Because these data are out of line with the results of other microbiological assays and of the chick tests they were not included in the averages.

Logarithmic plotting.—In reply to a question whether collaborators considered logarithmic plotting (as suggested by Wood (9)) an aid in evaluating, criticizing, and calculating data, differing opinions were expressed. Seven find logarithmic plotting an aid, three do not consider it helpful, and several made no comment. Observations typical of those expressed by others are quoted here: The chemist reporting from Laboratory 8 says, "It is of value in criticizing data but of little benefit for calculation. The characteristics of some turbidimeters place a break in the turbidity curves, even with logarithmic plotting." The person reporting for Labora-

tory 9 remarks, "Arithmetic grid paper gives satisfactory curves, is easy to work with, easier to read, and cheaper to buy. Perhaps a person thoroughly accustomed to working with logarithmic plotting might find it simpler but I do not." From graphs sent in by collaborators and from graphs plotted by the Associate Referee from the collaborator's data, it appears that acidimetric data, almost without exception, can be criticized easily when plotted on a log-log grid; turbidimetric data can very frequently be evaluated very easily when plotted on such coordinates. However, regardless of the type of graph paper used, the graphical representations sent in and the calculations made by the persons submitting results were without exception of unquestioned excellence and beyond criticism.

SUMMARY

The microbiological method for determining folic acid was subjected to collaborative assay in 1948. Chick tests on three of the four samples were made in five laboratories, as a check on the microbiological methods. Results showed excellent agreement among the laboratories.

Whether *L. casei* or *S. faecalis* was used in the medium suggested by the Associate Referee, and whether response of the test organism to the vitamin was measured acidimetrically or turbidimetrically, assay results were very acceptable.

The use of either of the bacteria in other media, measuring response either acidimetrically or turbidimetrically, gave results in harmony with those found in the medium suggested by the Associate Referee.

Folic acid values as determined by chick tests were higher than values obtained by microbiological assays. These findings emphasize again the need for further study of methods of extraction of the vitamin, and the possible influence of unidentified growth stimulants. New knowledge seems essential before results from microbiological assays will duplicate the results from chick tests.

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REPORT ON NICOTINIC ACID (NIACIN) OR NICOTINAMIDE (NIACINAMIDE)

MICROBIOLOGICAL METHOD

By HENRY W. LOY, Jr. (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Associate Referee**

The 1945 U.S.P.—A.O.A.C. official, first action, method for nicotinic acid, as described in *This Journal*, 30, 82 (1947), has been revised, and it is recommended† that this revised method be approved. The details of the method as revised are published in *This Journal*, 32, 110 (1949).

REPORT ON VITAMIN C (ASCORBIC ACID)

By WALLACE L. HALL (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

RECOMMENDATIONS‡

It is recommended—

That the official, first action, method for vitamin C (36.47, 36.48) be made official, final action, after the following minor corrections and additions are made:

(1) Add to the first sentence after "ferrous Fe," "stannous Sn and cuprous Cu."

(2) Change under REAGENTS (a), second line, "freshly pulverized stick HPO_3 " to read, "glacial HPO_3 , pellets, or freshly pulverized stick."

(3) Change "Note" to read as follows: "Products containing ferrous Fe, stannous Sn, and cuprous Cu give values in excess of their actual ascorbic acid content by this method."

Following are simple tests to ascertain whether these reducing ions are present in appreciable quantities to invalidate analysis: Add 2 drops of 0.05% H_2O soln of methylene blue to 10 ml of freshly prepared mixture of juice and the HPO_3 -acetic acid reagent, mix. Disappearance of methylene blue color in 5–10 seconds indicates presence of interfering substances. Stannous Sn does not give the test and may be tested for by using another 10 ml sample soln to which 10 ml of 25% HCl is added, mix, then 5 drops of 0.05% H_2O soln of indigo carmine, mix. Disappearance of indigo carmine color in 5–10 seconds also indicates presence of interfering substance.

No report was given on vitamin D—poultry.

* Appointed to succeed Frank M. Strong, resigned.

† For report of Subcommittee B and action of the Association, see *This Journal*, 32, 48 (1949).

‡ For report of Subcommittee A and action of the Association, see *This Journal*, 32, 48 (1949).

REPORT ON VITAMIN D

By CHESTER D. TOLLE (Division of Vitamins,* Food and Drug Administration, Federal Security Agency, Washington, D.C.),
Associate Referee

VITAMIN D IN MILK

The method for vitamin D in milk was proposed in 1937, and after collaborative study, was adopted in 1938. The procedure as it appears in the Sixth Edition of *Methods of Analysis* has been found entirely satisfactory and it is generally used in the assay of vitamin D in both fluid and evaporated milk. It is important to retain this method in the next edition of *Methods of Analysis*.

RECOMMENDATION†

It is recommended that the method for determining vitamin D in milk, which appears in the Sixth Edition as a tentative procedure (36.49–36.60, p. 621–625), be made official, first action.

REPORT ON CAROTENE

By F. W. QUACKENBUSH (Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Lafayette, Indiana), *Associate Referee*

Since the dropping of the phasic separation method for the determination of carotene in 1946, the Association has had no official method for the determination of carotene in foods and feeds. Two tentative methods have been introduced, one in 1946 and one in 1947. The objective this year was to compare these two tentative methods in collaborative study to determine whether one might be sufficiently satisfactory to be considered for adoption as an official method. A third method was also included for comparison. This method included two features which had been requested by several laboratories, namely, cold extraction and chromatography with a lower concentration of acetone.

Procedure I was the tentative procedure introduced in 1946, *This Journal*, 30, 84 (1947).

Procedure II was the one introduced in 1947, *Ibid*, 31, 111 (1948).

Procedure III was essentially the procedure of Silker, *et al.*, *Ind. Eng. Chem. (Anal. ed.)* 16, 513 (1944). The details sent to collaborators were as follows:

Extraction.—Weigh accurately a 1–2 gram sample into an Erlenmeyer flask or sample bottle, add 60 ml of a mixture of 1 part acetone and 2 parts hexane (Skellysolve B). Shake mixture, stopper container tightly, and set in the dark for 16–

* E. M. Nelson, Chief.

† For report of Subcommittee A, and action of the Association, see *This Journal*, 32, 48 (1949).

18 hours, usually overnight. Filter extract thru a Büchner funnel and wash the residue thoroly by decantation with several portions of hexane. Heat extract on a steam cone to drive off most of remaining acetone and to concentrate the soln to a volume of ca 40 ml.

This concentration is usually accomplished in 15 minutes. The entire solution is now chromatographed.

Chromatography.—Prepare a column and chromatograph the soln as in Procedure II, but use a 1:24 mixture of acetone and hexane instead of 1:9 for elution of the carotene. Collect the carotene eluate, make up to volume with 1:24 mixture of acetone and hexane, and determine the carotene concentration as directed under Procedure II.

Directions were sent out to a large number of laboratories and samples were mailed to each about two weeks later. Analyses were to be post-marked not later than ten days after the mailing date of the sample, and collaborators were instructed to hold samples at room temperature prior to analysis. Two samples of alfalfa meal were sent to each of the collaborators. One was a comparatively fresh dehydrated sample, the other had been in storage for a long period and had undergone considerable deterioration in carotene. The Referee is indebted to Herbert Schaefer, of the Ralston Purina Company, for packaging and distributing the samples.

COLLABORATORS

State and federal laboratories.—California, Indiana, Louisiana, Maine, Michigan, New Hampshire, North Dakota, Ohio, Oregon, South Dakota, Texas, Utah, Washington; Botany Laboratory, University of Chicago; Kansas State College; Utah State Agricultural College; Eastern, Southern, and Western Regional Laboratories; U. S. Food and Drug Administration, Washington, D. C.; Fish and Wild Life Service, Laurel, Maryland.

Industrial laboratories.—Wirthmore Research Laboratory, Malden, Mass.; The Best Foods, Inc., Bayonne, N. J.; Coop. G. L. F. Exchange, Inc., Buffalo, N. Y.; Eastern States Farmers' Exchange Incorporated, Buffalo, N. Y.; Central Mills, Inc., Dunbridge, Ohio; General Mills, Inc., (Larrowe Division), Rossford, Ohio; General Biochemicals, Inc., Chagrin Falls, Ohio; B. F. Goodrich Chemical Company, Akron, Ohio; M. F. A. Milling Company, Springfield, Mo.; Cerophyl Laboratories, Inc., Kansas City, Mo.; Ralston Purina Company, St. Louis, Mo.; National Alfalfa Dehydrating and Milling Company, Lamar, Colo.; Laucks Laboratories, Inc., Seattle, Wash.; Ayerst, McKenna and Harrison Limited, Montreal, Quebec.

SUMMARY OF SELECTED COMMENTS OF COLLABORATORS

The willing cooperation of the various collaborators is gratefully acknowledged. The individual comments were helpful in giving the full comparison of the procedures and it is regretted that space will not permit the full statement of each. Some of the most pertinent statements are quoted below.

Coll. No. 1.—In Procedure III it was necessary to increase the acetone concentration for satisfactory elution. A 2:23 acetone-hexane mixture was used.

Coll. No. 2.—A mixture of magnesium oxide and magnesium carbonate was used in Procedure I.

Coll. No. 5.—We believe that the presence of stereoisomers of *beta*-carotene should eventually require reinvestigation of the applicability of the absorption coefficient 196 at 435 millimicrons.

Coll. No. 8.—In Procedure II 50 ml. of acetone-hexane mixture was insufficient to wash the carotene through the absorbent. At least 100 ml was required.

Coll. No. 9.—Procedure II is susceptible to giving excessive values through too strong an eluting action.

Coll. No. 11.—We do not believe that any one method has all the features desirable in an official method. Each method has, we feel, one or more steps which need further study. As now written, however, our choice would be for Method No. 2.

Coll. No. 14.—No Magnesia #2642 available. Used #2641 in Procedures II and III.

Coll. No. 15.—Satisfactory separation of pigments could not be obtained by magnesium carbonate as adsorbent.

Coll. No. 16.—Procedure I should be abandoned for studies on dehydrated leaf material. It is far too laborious.

Coll. No. 23.—In Procedure III the 1:24 acetone-hexane would not elute carotene.

Coll. No. 25.—We had difficulty in elution of the carotene with 1:24 acetone-hexane.

Coll. No. 31.—Procedure II seems most adaptable to routine or control analyses. An overnight wait is undesirable in a control method and in this case gave lower results in our hands. Procedure I has many of the cumbersome separatory funnel operations of earlier procedures which it is desirable to avoid.

Coll. No. 32.—In Procedures II and III, using Westvaco 2642 magnesia 1:1 with Supercel, we find the carotenes pass through very slowly.

Coll. No. 34.—Certain lots of Hyflo Supercel will retain more than 5 per cent of carotene.

RESULTS AND DISCUSSION

The experimental results, received from thirty-five laboratories, are summarized in Table 1

Procedure I gave the lowest average results. As pointed out by some collaborators, conditions used in this procedure favor isomerization. Isomers such as neo-*beta* carotene B with maxima shifted to lower wave lengths would predominate, thus resulting in low values when read at 450 millimicrons and calculated with the extinction coefficient of 2580. Actually, Collaborator 14 found the results to be 12 per cent higher when his solutions from Procedure I were read at 436. This evidences the high degree of isomerization in Procedure I. If 12 per cent were added to the average results of Beckman users for this procedure, the results would be 38 for the old sample and 131 for the fresh sample, which are probably nearly correct.

Procedure II gave the highest results. One collaborator (No. 9) reported that the acetone content of the solution during chromatographing was high enough to elute non-carotene pigments. However, studies in the Referee's laboratory do not substantiate this and other collaborators do

TABLE 1.—*Results of analysis of alfalfa meal samples*

COLL. NO.	INSTRUMENT	FRESH SAMPLE			OLD SAMPLE		
		PROCEDURE—			PROCEDURE—		
		I	II	III	I	II	III
1	Beckman-DU	37	34	32	122	123	123
2	"	31	51	37	115	133	124
3	"	32	36	30	115	128	117
4	"	33	39	21	119	115	101
5	"	35	38	35	119	129	130
6	"	39	36	36	122	118	125
7	"	26	47	30	106	140	115
8	"	29	38	36	117	131	131
9*	"	20	73	41	92	340	126
10	"	—	32	33	—	129	136
11	"	29	35	32	110	124	115
12	"	—	—	—	—	120	128
13	"	48	45	38	127	143	133
14	"	30	44	38	102	146	111
15	"	44	48	31	99	164	164
16	"	—	39	39	—	136	142
17	"	35	34	35	129	131	129
18	"	—	36	32	—	136	125
19	"	42	39	30	124	129	124
20	"	36	42	—	124	135	—
21	"	34	33	37	120	119	118
22	"	34	39	31	123	129	128
23	"	34	31	—	120	119	—
24	"	27	47	38	96	136	125
25	"	—	34	—	—	124	111
Averages, Beckman		34	39	34	117	131	125
26	Coleman-11	33	37	32	136	135	123
27	"	32	34	33	104	113	121
28	"	39	41	35	141	140	122
29	"	34	37	32	120	128	131
30	Evelyn	39	39	38	138	137	140
31	"	35	39	32	127	135	126
32	"	38	36	36	140	138	132
33	Cenco-Sheard	40	48	41	108	150	135
34	Cenco	37	34	34	132	124	119
35	Klett-Sum- merson	32	41	—	142	130	—
Averages, other instru- ments		36	39	35	129	133	128
Averages, all values		35	39	34	120	131	126

* Values not included in averages.

not seem to have experienced such difficulty. Some indicated preference for the use of a thimble in the extraction. In our experience this necessitates a much longer period of heating to effect complete extraction and results in a substantial increase in isomerization of the carotene.

Procedure III gave lower results than Procedure II in twenty cases, equal or higher results in eleven cases. Several collaborators expressed doubt on the stability of carotene solutions in the desk overnight. Four collaborators who obtained low values stated that the 4 per cent acetone solution did not elute all of the carotene. Three of these considered their results unreliable and did not report them.

To the question "Do you feel that any of the three procedures should be adopted by the A.O.A.C. as official?" twenty recommended that Procedure II be adopted, three others voted for either Procedure II or III, three expressed preference for Procedure II but did not recommend official adoption, one recommended Procedure III only, one preferred Procedure I, four recommended other procedures than those used in the collaborative study, and four offered no opinion. In view of the recommendation by a large majority it is felt that Procedure II should be adopted as official, first action, for hays and dried plants.

The instrument used for analysis of the carotene fraction following chromatography apparently had little influence on the result, since the averages of twenty-five values obtained with Beckman spectrophotometers agreed well with the averages of ten values obtained with other instruments, except in the case of Procedure I. This difference possibly reflects the error mentioned above, of measuring alkali-treated carotenes at 450 millimicrons. Further efforts should be made to work out analytical procedures which will not produce isomerization. Until such time as this is accomplished total carotenes must be measured. The high selectivity of the Beckman instrument is probably not advantageous for the routine measurement of total carotenes in chromatographed extracts from leafy materials. Less costly equipment should serve the purpose equally well.

RECOMMENDATIONS*

It is recommended—

(1) That Procedure II (*This Journal*, 31, 111, 1948), which was adopted in 1947 as an alternative tentative method, be adopted as official, first action, for analysis of hays and dried plants.

(2) That Procedure I (*This Journal*, 30, 84, 1947) be discontinued as a method for hays and dried plants, but that it be continued as a tentative method of analysis for other materials.

(3) That studies on analysis for carotene be continued.

No report was given on pantothenic acid.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 48 (1949).

REPORT ON PRESERVATIVES AND ARTIFICIAL SWEETENERS

By MARGARETHE OAKLEY (State Department of Health, Baltimore 18, Md.), *Referee*

The chapter on Preservatives and Artificial Sweeteners got off to an auspicious start this year with requests for the assignment of ten topics for study and the final assignment of nine of these. No Associate Referee was obtained for the sweetener 1-propoxy 2-amino 4-nitrobenzene.

Saccharin.—Early in the year the Associate Referee on saccharin asked to be relieved of the assignment because of the pressure of routine work and requested that it be transferred to W. S. Cox of Atlanta, who is at present interested in both saccharin and dulcin but is not doing A.O.A.C. work on them.

Dulcin.—No report was received from the Associate Referee on dulcin. With the increase in sugar supplies the urgency of the problem has passed; however, it is deemed advisable to continue with this work, especially since the present method has been so roundly condemned by those called upon to refer to it.

It is therefore recommended that both dulcin and saccharin be reassigned for study during the coming year.

Quaternary Ammonium Compounds and Monochloroacetic Acid.—Methods for determining quaternary ammonium compounds and monochloroacetic acid were completed last year. The work for the current year was to extend the application of these methods to a greater variety of foods and to study further details of the methods collaboratively.

Dichloroacetic Acid.—No report on dichloroacetic acid was submitted, the Associate Referee being unable to find time this year to devote to the problem. This subject was covered very ably and thoroughly by Charles F. Bruening (*This Journal*, 28, 620 (1945)). He presented a procedure for the quantitative determination of dichloroacetic acid in food products involving its isolation by an ether extraction and the conversion of the chloride of the acid to the ionic form by ignition with sodium carbonate. The chloride thus formed was determined by the Volhard method. In the same article Mr. Bruening gave an identification test for dichloroacetic acid involving the preparation of the p-toluide derivative of the acid which is characterized by a melting point determination.

With this work as background it was thought that a little collaborative work would be all that was necessary to complete the problem. However, since the methods for extraction and determination are the same for mono and dichloroacetic acids and it has been submitted to collaborative study with monochloroacetic acid, the identification test for dichloroacetic acid is the only part of this problem remaining. In view of the extremely slight need for this identification test and since the work of Mr. Bruening

is available in *The Journal* for those who do need it; and considering the policy of pruning the *Methods of Analysis* to the very essentials, it is therefore recommended that the subject of dichloroacetic acid be dropped for the present.

Thiourea.—The Associate Referee has studied a short oxidation method for thiourea before its reaction with Grote's reagent and has already submitted it to collaborative study. He is enthusiastic about the results of his study and recommends its substitution for the present tentative method.

Formaldehyde.—After a review of the many tests for formaldehyde in the Book of Methods, and elsewhere, the Associate Referee offered many recommendations including the deletion of three present methods. Two other present methods he recommends for collaborative study and to all of these he adds one modified and two new methods which he recommends for collaborative study. The Referee concurs and hopes that the final outcome of these endeavors will produce one or two satisfactory procedures to replace the present seven antiquated ones.

Esters of Benzoic and Vanillic Acids.—The Associate Referee worked on the recovery of ethyl vanillate from orange and tomato juices. He used an extraction method, determining the vanillic acid by actual weight. The method proved satisfactory in his hands but he does not recommend submitting it to collaborative study. Information received by him indicates that ethyl vanillate is not being manufactured commercially and under those circumstances he feels it cannot be a regulatory problem at present.

The questions with regard to this Associate Refereeship are: (1) Are there any hydroxy benzoates being sold in the United States at present? and (2) Are the methods for benzoates in the chapter on Preservatives in *Methods of Analysis* adequate for their determination?

Formic Acid.—There has been some discussion relative to the advisability of eliminating the formic acid method from the chapter on Preservatives and Artificial Sweeteners, putting in its place a reference to the formic acid determination along with the other volatile acids. The Referee recommends the appointment of an Associate Referee on formic acid to study the subject, to confer with Mr. Hillig concerning elimination of duplication in the *Methods of Analysis* of the formic acid determination, and also to investigate the colorimetric method for the detection of formic acid which appeared in *Analytical Chemistry*, 19, 206-7 (1947).

Mold Inhibitors—Propionates.—The Associate Referee concentrated his efforts during the past year on determining the loss of propionates and diacetates during baking and subsequent air drying of bread. He recommends determination on the fresh basis and warns that acetic acid is present normally in bread in amounts that must be taken into consideration

in the detection of added acetic acid or sodium diacetate. He further recommends that an alternate chromatographic technic for the identification and estimation of volatile fatty acids in bakery products be undertaken.

RECOMMENDATIONS*

The Referee concurs in the recommendations of the Associate Referees on preservatives and artificial sweeteners which have been presented.

REPORT ON BENZOIC AND VANILLIC ACID ESTERS

By W. J. MCCARTHY (Food and Drug Administration, Federal Security Agency, Cincinnati 2, Ohio), *Associate Referee*

During the past year experimental work on the recovery of ethyl vanillate from orange juice and tomato juice was carried out on solutions of the juices made up to contain 0.1 per cent ethyl vanillate. For this work a stock solution of ethyl vanillate was prepared by dissolving the required amount in a slight excess of normal sodium hydroxide solution and then adding distilled water to desired volume.

The method of analysis used on these samples was the general outline as described in the previous report October 1947,** except that chloroform was used as solvent instead of ether, and any alcohol formed on hydrolysis was not removed. Recovery of ethyl vanillate from the orange juice preparation was 95.4 per cent, and from the tomato juice 94.6 per cent. The vanillic acid was determined by actual weight along with melting point.

In view of the fact that ethyl vanillate is not manufactured commercially and hence is not being used in food products, the above method has not been subjected to collaboration.

It was noted in last year's report that ethyl vanillate was not available commercially, and recent information from the Institute of Paper Chemistry, Appleton, Wisconsin, shows that the same situation exists today.

Since the use of ethyl vanillate as a preservative for food is not a current problem, and in view of the workable method herein described, it is recommended† that work on this subject be discontinued until a regulatory problem exists.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 57 (1949).

** *This Journal*, 31, 478 (1948).

† For report of Subcommittee C and action of the Association, see *This Journal*, 32, 57 (1949).

REPORT ON QUATERNARY AMMONIUM COMPOUNDS
IN FOODS

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

This year the Associate Referee made up samples of thirteen foods by adding quaternary ammonium compounds to them and submitted them to nine collaborators. The samples could not be sent out until the latter part of July because of difficulties encountered in obtaining some of the groceries which had been selected for study. So far, complete reports have been submitted by only three collaborators and partial reports from three others. The Associate Referee feels that more time should be allowed the collaborators and is postponing the report on these samples for another meeting of the Association. The foods used fall into five groups, as follows:

1. Beverages: Root beer, creme soda, imitation grape soda.
2. Dressings: Mayonnaise, French dressing, sandwich spread.
3. Milk: Two water solutions to be added to milk.
4. Relish: Chopped pickle relish with the addition of quaternary ammonium compounds at two levels.
5. Fruit Juices: Apple, tomato, orange.

It is hoped that the remaining collaborators will have an opportunity to complete the analyses during the coming year and have reports ready before the next meeting.

RECOMMENDATIONS*

It is recommended—

(1) That the ferricyanide method for quaternary ammonium compounds in commercial preservatives, *This Journal*, 31, 105 (1948), be made official, final action.

(2) That the method for table sirup, *Ibid.*, 31:108 (1948) be made official, final action.

(3) That the method for bottled beverages containing fruit juices, *Ibid.*, 31, 106 (1948), be made official, final action.

(4) That the method for beer, *Ibid.*, 31, 108 (1948), be made official, final action.

(5) That collaborative study be continued on the following methods:

(a) Method for Fruit Juices, *Ibid.*, 29, 318 (1946).

(b) Shorter Method for Fruit Juices, *Ibid.*, 29, 319 (1946).

(c) Method for Bottled Sodas, *Ibid.*, 29, 323 (1946), subject to increasing the volume of bromophenol blue reagent to 5–10 ml.

(d) Method for Milk, *Ibid.*, 29, 324 (1946), on samples containing preservative quantities of quaternary ammonium compounds.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 57 (1949).

- (e) Method for Mayonnaise, Salad Dressings, and Sandwich Spreads, *Ibid.*, 29, 323 (1946).
- (f) Method for Pickles and Relishes, *Ibid.*, 29, 326 (1946).

REPORT ON MONOCHLOROACETIC ACID

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

INTRODUCTION

Collaborative samples 5-10¹ were submitted to the collaborators listed, with the instruction given below.

Sample 5 was a commercial preservative.

Sample 6 consisted of canned orange juice to which a weighed amount of monochloroacetic acid was added and mixed in a five-gallon bottle. The mixture was filled into quart bottles which were then heated at 100°C. in an "Arnold Steam Sterilizer" for 30 minutes with the screw cap barely loose to permit the escape of air.

Samples 7, 8, and 9 consisted of Orange Soda, Imitation Strawberry Soda, and Beer, respectively. In these cases water solutions of monochloroacetic acid were prepared containing such quantities of the chemical that when 5 ml of the solution was pipetted into a bottle of the product it would impart the desired amount of the preservative. As these products were all carbonated and closed with crown caps, they were cooled in a freezing room for several hours. When cold the crown cap was removed, 5 ml of the appropriate solution added with a pipet, the bottle recapped at once, and mixed.

In the preparation of Sample 10 a weighed quantity of monochloroacetic acid was dissolved in 16 liters of red wine and after thorough mixing, filled into quart bottles closed with screw caps.

DIRECTIONS FOR COLLABORATORS (1948)

I. Efficiency of Extractors.

Before proceeding with the analysis, determine the efficiency of your extractors as follows:

Prepare a solution containing 1 gram of monochloroacetic acid per liter. Following the method, extract several aliquots (100 ml, 50 ml, 25 ml) diluted to 100 ml with water, for various lengths of time (2 hrs, 3 hrs, 4 hrs, etc.), hydrolyze and determine Cl in order to find the time needed to obtain a recovery of 95 % or more.

II. Sample 5. Preservative.

(a) Pipet a portion of sample into a volumetric flask, make up to the mark with water and determine monochloroacetic acid in duplicate, in aliquots containing 1 to 2 ml of the original sample, by methods 1 and 2 below. Report as mg CH₂Cl COOH per 100 ml.

Method 1. Follow *This Journal*, 31, 104 (1948).

Method 2. To 100 ml of sample add 3 ml of H₂SO₄ and shake in a separatory

¹ For last year's report, using 4 samples, see *This Journal*, 31, 484 (1948).

funnel with three equal volumes of ether. Unite the ether extracts and wash by shaking with two 30 ml portions of 1 *N* NaOH. Hydrolyze for 2 hrs on the steam bath and determine Cl as in Method 1.

Barium Test

(b) Dilute 4–5 ml of sample to 100 ml, add 6 ml of H_2SO_4 (1+1), and extract with an equal volume of ether in separatory funnel. In cases where emulsions form, extract in a continuous extractor for 1 hour. Transfer the ether extract to a separatory funnel, add a few drops of phenolphthalein indicator, 5 ml of ± 0.1 *N* $\text{Ba}(\text{OH})_2$, and shake for 30 seconds. If the water layer takes on the pink color of phenolphthalein, transfer thru a filter paper to a small beaker. Add ± 0.05 *N* acetic acid until colorless and evaporate to 1–2 ml on the steam bath. Allow the remaining liquid to evaporate spontaneously in the air and finally in a desiccator. If 5 ml of $\text{Ba}(\text{OH})_2$ does not give a pink water layer, add 5 ml more before separating. Repeat the extraction with $\text{Ba}(\text{OH})_2$ several times or until a pink soln is obtained, evaporating each barium soln in a separate beaker. Examine the crystals under the polarizing microscope.

Optical-Crystallographic Properties of Barium Monochloracetate

Barium monochloracetate monohydrate crystallizes from water in plates, many of which are hexagonal in habit and frequently forming in overlapping layers. Even in material that has been finely powdered for microscopic examination, the pointed terminations of the plates, often in pairs, can be observed. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative on the more elongated plates. The plates invariably extinguish sharply with crossed nicols and therefore interference figures were not observed in convergent polarized light (crossed nicols). In view of the fact that the plates persistently lie in one orientation the significant refractive indices were determined by the statistical method, measuring the lowest and highest indices, respectively, on plates showing the maximum amount of double refraction. These two indices are therefore arbitrarily designated as n_α (the minimum value) and n_γ (the maximum value). The two significant refractive indices are: $n_\alpha = 1.582$ and $n_\gamma = 1.611$, both ± 0.002 , and frequently shown on the platy fragments.

Solutions containing dichloroacetic acid yield a barium compound that produces a vitreous residue, which is hygroscopic. A crystalline barium trichloroacetate is formed under the conditions set forth, but so far it has not been found suitable for microscopic identification although it crystallizes in a habit distinctly different from barium monochloracetate.³

(c) Barium-Indigo Test

REAGENTS

- (1) *Anthranilic acid reagent*.—Dissolve 1 g anthranilic acid in 40 ml of H_2O , add 0.3 g NaOH, and make up to 50 ml.
- (2) *Caustic soda soln*.—Dissolve 10 g NaOH in 10 ml H_2O and filter if necessary.

PROCEDURE

Dissolve 0.17 g of the barium salt in 5 ml H_2O in a 10 ml graduate, add 1.05 ml of 1.0 *N* H_2SO_4 , make up to 10 ml, and mix. Let stand until the precipitate settles or filter if preferred. Pipet 3 ml of the clear liquid into a small beaker, add 2 ml anthranilic acid reagent and 30 mg Na_2CO_3 (weighed). Test with litmus paper.

³ NOTE: Have a field chemist familiar with the procedure make the microscopic examination of the crystals (*This Journal*, 27, 447 [1944]), and send any remaining crystals to the Microchemical Section for check analysis.

If acid, add one additional 30 mg of Na_2CO_3 . Pour into test tube and heat in water bath for $\frac{1}{2}$ hour. Place the test tube in an oven at $125^\circ \pm 5^\circ\text{C}$. until only a moist residue remains. Remove from the oven, add 2 drops of caustic soda soln directly upon the residue. (If the residue is entirely dry, add 1–2 drops H_2O and let stand until absorbed by the residue before adding the strong NaOH), return to the oven until completely dry (at least 1 hour). Remove from the oven and heat the test tube at $310^\circ\text{--}320^\circ\text{C}$.¹ until the contents assume an orange color. (This requires 15 seconds to 2 min., but must be carefully watched to remove from the heat as soon as the reaction is complete). Cool slightly, add 5–7 ml H_2O from a wash bottle, splashing the water to incorporate air into it. Warm over a flame and blow air thru the soln 1–2 min. using a pipet or glass tube. Heat to boiling over the flame and again blow air thru the soln. (As the oxidation progresses, the soln turns red if monochloroacetic acid is present, then green or blue or a combination of the two, and finally solid particles of indigo separate out. They have a tendency to rise to the surface at first.) Let the mixture stand about 10 min., then acidify slightly with HCl (1+1). After standing further for $\frac{1}{2}$ hr. filter and wash the precipitated indigo with water to remove acid. Allow the paper to dry in the air and preserve as an exhibit.

Indigo Test

(d) Dilute 2 ml of sample to 100 ml, add 3 ml of H_2SO_4 and shake with 100 ml of ether. Add 3 ml of anthranilic acid reagent to the ether extract, evaporate at a low temp., filter off any insoluble matter and apply the indigo test as under (c) beginning "Test with litmus paper, etc."

Pyridine Test

(e) Extract 2 ml of sample as under (d) and treat the ether extract by the method in *This Journal*, 29: 104 (1946) under the heading "Qualitative method applicable to Beverages and Fruit Juices" parts 1 and 2.

III. Samples 6 to 10, inclusive.

(a) Mark the contents level of the bottles containing samples 7, 8, and 9 so that they may be measured subsequently and report the contents measured.

(b) Determine monochloroacetic acid in duplicate in 100 ml of sample by methods 1 and 2 above, p. 489. Report as mg $\text{CH}_2\text{Cl COOH}$ per 100 ml, and in case of Samples 7, 8, and 9 also report as mg $\text{CH}_2\text{Cl COOH}$ per bottle.

PREPARATION OF SAMPLE

(c) Acidify two 100 ml portions with 3 ml of H_2SO_4 and extract, using either continuous extractors or separatory funnels (state which is used) and apply the indigo test to one extract and the pyridine test to the other.

1. *Indigo test*.—Add 3 ml of anthranilic acid reagent to the ether extract and evaporate at a low temp. If any insoluble matter (oily or solid) separates out, filter the remaining liquid thru a small wet filter paper into a 50 ml beaker. If no insolubles come out, transfer the residue to beaker. Now apply the test as described under II (c) "Barium-Indigo Test" beginning "Test with litmus paper, etc."

2. *Pyridine test*.—Treat the extract by the method in *This Journal* 29: 104 (1946) under the heading "Qualitative Method applicable to Beverages and Fruit Juices," parts 1 and 2.

(d) In the case of Samples 9 and 10 apply the Mallory-Love procedure to 100 ml of sample. *Ind. Eng. Chem., Anal. Ed.*, 15: 492 (1943).

¹ For the fusion at $310\text{--}320^\circ\text{C}$ use a brass block having a well to contain the test tube and a second well to contain a thermometer. The block is wrapped with a coil of nicrome wire and the heat controlled by a variable voltage transformer. Analysts have used muffle furnaces, micro-burners, Wood's metal, or solder baths, etc., for the fusion with equal success.

The following persons collaborated in these tests: H. M. Bollinger, Los Angeles, Calif.; Harry W. Conroy, Kansas City, Kans.; Joyce Holberg, Minneapolis, Minn.; Gardner Kirsten, New York, N. Y.; D. W. McLaren, Buffalo, N. Y.; Angus J. Shingler, Atlanta, Ga.; John B. Wilson, Washington, D. C. All were members of the staff of the U. S. Food and Drug Administration, Federal Security Agency.

Since most of the collaborators reported the results obtained in the determination of the efficiency of their extractors, they have been brought together in Table 1.

TABLE 1.—*Efficiency of extractors used on monochloroacetic acid*

COLLABORATOR	TIME		
	PRESENT	2 HRS.	3 HRS.
	mg	per cent	per cent
H. M. Bollinger	25		97
	50	92	
	100	93	99
Harry W. Conroy			Over 95
Joyce Holberg	25	94	97
	50	92	94.5
	100	87.5	97
D. W. McLaren ¹	25	102.3	102.3
	100	99.7	99.7
Angus J. Shingler	25	94.0	99.2
	50	97.6	99.2
	50	98.6	99.2
	100	93.5	99.0
John B. Wilson	25	90.8	95.2
	50	94.6	99.2

¹ Extractors used by this collaborator are made of tubing 1 inch in diameter. They are about 47 inches in length, with the delivery tube 30 inches from the bottom. The 100 ml of sample was diluted with 100 ml of water for the extraction.

The results obtained with quantitative methods 1 and 2 (which differ as to mode of extraction) are given in Table 2. The average recoveries by both methods are well above 90 per cent for all samples except the orange juice.

QUALITATIVE TESTS

The collaborative results on the qualitative tests are given in Table 3.

These results warrant the adoption as official, first action, of the following qualitative tests:

TABLE 2.—*Monochloroacetic acid (Quantitative Methods 1 and 2)*

SAMPLE NO. PRODUCT	(5) PRESERVATIVE		(6) ORANGE JUICE		(7) ORANGE SODA		(8) STRAWBERRY SODA		(9) BEER		(10) WINE	
	g/100 ml	g/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml
Present	5.44	5.44	22.7	22.7	13.0	13.0	11.0	11.0	14.4	14.4	19.0	19.0
Found by Method	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Collaborator:												
H. M. Bollinger	5.24	5.22	20.3	17.8	14.2	13.8	11.0	11.3	14.5	13.4	18.5	18.5
	5.22	5.20	19.0	19.5	14.2	13.5	11.0	10.9	14.0	14.0	18.2	18.3
Harry W. Conroy	5.28	5.27	18.4	16.5 ¹	13.1	12.8	10.9	10.2	13.9	13.3	18.0	17.9
	5.27	5.27	18.0	16.0 ¹	12.9	12.8	10.4	10.4	13.7	13.2	17.8	17.7
Joyce M. Holberg	5.15	5.08	24.0	17.3 ¹	11.8	10.3	10.1	10.5	14.5	13.5	13.0 ²	20.4
	5.13	5.23	23.3	17.8	11.3	11.3	9.4	10.0	14.3	13.3	10.0 ²	21.0
Gardner Kirsten	5.30	5.21	19.1	— ¹	12.8	12.7	11.0	10.5	14.9	— ¹	18.7	— ¹
	5.30	5.22	18.6	— ¹	12.5	11.8	11.1	10.1	14.8	— ¹	18.4	— ¹
D. W. McLaren	5.42	5.39	16.3	14.4 ¹	13.1	10.9	11.4	10.4	13.2	12.7 ¹	16.7	17.5
	5.42	5.24	19.2	15.8 ¹	12.8	11.1	10.6	9.9	11.1	13.2 ¹	17.2	17.7
Angus J. Shingler	5.23	4.83	18.0	15.7 ¹	12.7	12.4	11.4	10.6	13.9	15.0 ¹	17.0	17.7
	5.26	4.49	18.0	18.5	12.2	12.4	11.4	10.6	14.2	13.1 ¹	17.7	17.5
John B. Wilson	5.43	5.22	23.0	17.4 ¹	12.9	12.3	11.2	10.6	14.0	13.4	19.0	18.5
	5.42	5.38	21.0	18.2 ¹	12.3	11.8	10.8	10.1	14.3	14.0	18.5	17.9
Maximum	5.43	5.39	24.0	19.5	14.2	13.8	11.4	11.3	14.9	15.0	19.0	21.0
Minimum	5.13	4.49	16.3	14.4	11.3	10.3	9.4	9.9	11.1	12.7	10.0	17.5
Average	5.29	5.15	19.7	17.1	12.8	12.1	10.8	10.4	13.9	13.5	17.2	18.4
Average recovery, per cent	97.2	94.6	86.8	75.3	98.5	93.1	98.2	94.5	96.5	93.8	90.5	96.8

¹ Emulsions formed and interfered with the determination.² If these two results are eliminated because of the obvious incomplete extraction, the minimum result becomes 16.7; the average 17.9; the average recovery, 94.2%.

A. For use on commercial preservatives:

- (1) The Barium Test, accompanied by microscopic identification of the crystals.
- (2) The Barium-Indigo Test.
- (3) The Pyridine Test.

B. For use on carbonated beverages, fruit juices, and beer:

- (1) The Indigo Test, as given in this report.
- (2) The Pyridine Test.

Several collaborators consider the Mallory-Love version of the indigo test too tedious and others lack confidence in it because of frequent fail-

TABLE 3.—*Monochloroacetic acid (qualitative tests)*

TEST	SAMPLE	B	C	G	H	K	McL	S	W
Barium	5	+ ¹	+ ¹		+ ¹	+ ²	+ ¹	+ ¹	+ ¹
Indigo Barium	5	+	+		+	+	+	+	+
Indigo	5	+	+		+	+ ³	+ ⁴	+ ⁴	+ ³
	6	+ ³	+ ⁴	+	+ ³	+	+	—	+
	7	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+
	9	—	+	+	+	+	+	—	+
	10	—	+	+	+	—	+	+	
Mallory-Love	9	—	+		+	+	+		+
	10	+	+		+	+	+		+
Pyridine	5	+	+		+	+	+	+	+
	6	+	+	+	+	+	+	—	+
	7	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+
	9	+	+	+	+	+	+	—	+
	10	+	+	+	+	+	+	+	+

¹ Crystals of barium monochloroacetate were identified microscopically by W. G. Helsel, Microanalytical Section.

² Crystals of barium monochloroacetate were also identified microscopically by C. A. Wood, New York, N. Y.

³ Extractions for these tests made with a continuous extractor.

⁴ Extractions for these tests made in separatory funnels.

ures to obtain the test when they felt certain that monochloroacetic acid was present in the sample being examined. However, the Associate Referee freely admits that no qualitative test for monochloroacetic acid has yet been devised which can be guaranteed against failure, so that he has reached the conclusion that while a positive test by any of the procedures studied is conclusive evidence of the presence of monochloroacetic acid, a negative test by any or all of them is no guarantee of the complete absence of monochloroacetic acid.

For instance, on frequent occasions it has been found that the barium monochloracetate crystals in the residues obtained from food samples were very much like the proverbial needle in a haystack. For this reason it has been decided to limit its use to commercial preservatives. There have been times, however, when the crystals could not be found but the Indigo test on the crystals was successful.

The Pyridine test has been subject to failure at times because of unknown impurities which interfere with crystallization of the betaine. However, most acid substances are eliminated if sufficient pyridine is added in the test. If more than 5 ml of normal sodium bicarbonate solution is required to extract the acids from the ether, as has happened frequently in this laboratory, there should be a corresponding increase in the quantity of pyridine added, in which case the acids distil off under diminished pressure with the pyridine.

The Indigo test is subject to failure because the dried mixture in the test tube may be so dark that the color changes during the fusion cannot be observed, and the analyst is not only deprived of seeing the color changes which show the presence of the chemical, but is even unable to tell when the requisite fusion period is over.

In the Mallory-Love version of this test there are three evaporations, (1) in a dish from ether and alcohol, (2) in a beaker from ether, and (3) in a beaker from benzene. Monochloroacetic acid may be lost at any of these points if the residue is allowed to go completely dry. The vessel must be removed from before the fan at just the right moment to have the test work perfectly.

Another danger point is in the extraction with benzene, since an excessive quantity of water at this point will cause enough loss of monochloroacetic acid in the water layer to vitiate the test.

Members of the Food and Drug Administration in Cincinnati, Chicago, and Buffalo have suggested procedures for purification of the ether extract by treatment with barium hydroxide and other means which the Associate Referee will investigate further for next year's work on the analysis of wine.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of monochloroacetic acid, *This Journal*, 31,104 (1948), be adopted as official, final action, for carbonated beverages.

(2) That the method for the determination of monochloroacetic acid, *Ibid.*, 31,104 (1948), be adopted as official, first action, for beer and wine.

(3) That the following parenthetical expression be added to the method for monochloroacetic acid in carbonated beverages, beer, and wine (*Ibid.*,

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 57 (1949).

p. 105) after the sentence ending "extract with ether 2-3 hours." "(Use the length of time found necessary for a recovery of at least 95 per cent when known quantities of monochloroacetic acid are extracted in the apparatus.)" (Official, first action.)

(4) That the following paragraph be added to the method for monochloroacetic acid: (*Ibid.* 104 (1948): "The following equally efficient means of extraction may be used:

"To 100 ml of sample add 3 ml of H_2SO_4 and shake in a separatory funnel with three equal volumes of ether. Unite the ether extracts and wash by shaking with two 30 ml portions of 1 N NaOH. Unite the two NaOH solutions and digest as above." Official, final action.

(5) That the following method be adopted as official, first action.

Determination of Monochloroacetic Acid in Commercial Preservatives

Prepare a dilution of the sample that will permit the measurement of a convenient aliquot containing 50-100 mg of monochloroacetic acid and determine in such aliquot as directed under the method for carbonated beverages.

(6) That the following qualitative tests for monochloroacetic acid in commercial preservatives be adopted as official, first action, as given above under "II. Sample 5." Barium Test; Barium-Indigo Test; Indigo Test; Pyridine Test.

(7) That the following qualitative tests for monochloroacetic acid in carbonated beverages, orange juice, beer and wine be adopted as official, first action, as given above under "III. Samples 6-10, inclusive." Indigo Test; Pyridine Test.

(8) That further work be done on the determination of monochloroacetic in fruit juices including orange juice.

(9) That further work be done on qualitative tests for monochloroacetic acid.

REPORT ON MOLD INHIBITORS

By LEWIS H. McROBERTS (Food and Drug Administration, Federal Security Agency, San Francisco, Calif.), *Associate Referee*

LOSSES OF PROPIONIC AND ACETIC ACIDS IN THE BAKING AND AIR DRYING OF BREAD

The previous report (1) outlined procedures for the detection of added propionates in bakery products. Samples of air dried white bread prepared with sodium propionate were submitted to collaborators for analysis by the method now adopted as "tentative" (2). These experiments disclosed a loss of propionate that apparently took place during baking and/or air drying in preparation of samples. The present investigation has been directed to determining the source or sources and the magnitude of this loss. The experiments were expanded to include the addition of acetic acid by means of the product "sodium diacetate" and to both white and

whole wheat breads. The fresh bread was analyzed to detect baking loss and the air dried bread to determine total loss by that method of sample preparation. The total loss less the apparent baking loss was then attributed to air drying.

BREAD PREPARATION

The bread for the experiments described in this report was baked in the cereal section laboratory of the U. S. Food and Drug Administration in Washington, D.C., by V. E. Munsey, and was forwarded by air express to this destination. The following described formula was used for both white and whole wheat breads:

Flour	315 gms.	Lard	10 gms.
Water	198 gms.	Sugar	12 gms.
Yeast	8 gms.	Salt	7 gms.
Dry Skim Milk	12 gms.		

The first baking consisted of nine one-pound, white loaves of which three were for control, three contained added "sodium diacetate" and three contained added sodium propionate. The inhibitors were added to the flour in the proportion of 0.25 per cent diacetate and 0.20 per cent propionate, or, respectively, 0.16 per cent and 0.12 per cent in the bread. This series of samples was then duplicated in whole wheat bread with the exceptions that the inhibitors were added to the dough for each loaf in amounts of 0.16 per cent of propionate or diacetate on the finished bread basis. The loaves were weighed one hour after baking and upon receipt at destination. Weight losses in transit were found to be 3-5 per cent. Analyses were started on the fresh bread in from one to two days following baking. The white bread was air dried for four days and the whole wheat bread for seven days at 23-25°C previous to grinding to 20 mesh in a steel burr mill.

Samples of the propionate-flour mixture, diacetate-flour mixture, wheat flour, sodium propionate, and "sodium diacetate" used in preparing the breads were analyzed to check the mixing of flour and inhibitor, the amount added, and the purity of the inhibitors as to propionic or acetic acids available.

SAMPLE PREPARATION

The three loaves of each batch were sliced to give about eighteen slices per loaf and divided into two composites by alternate slices. The composites were then weighed and designated as one portion to be analyzed fresh and one portion for air drying. From this point, subsequent preparation was as previously described (2).

METHOD

The tentative method for volatile fatty acids in bakery products (2) was used in the analysis of all samples. Flour mixtures with added inhibitors were treated the same as air dried bread. Sodium propionate or sodium diacetate were distilled directly on the basis of 500 mgs of the original inhibitor in the distillation flask.

RESULTS

(1) *Sodium Propionate*.—The propionate added in the preparation of the bread was labeled sodium propionate. In that the calcium salt is often used for the same purpose, tests were made for calcium with negative results. The pH of a 0.5 per cent solution was found to be 6.9. When distilled (3) from a solution made acid to Congo Red with sulphuric acid, propionic acid was identified by the standard "C" ratio for that acid, and by the formation of characteristic mercurous propionate crystals. No other volatile acids were detected. The purity as sodium propionate was calculated to be 98.5 per cent.

(2) "*Sodium Diacetate*."—The chemical sold under this name has been described as a "solid form of acetic acid." The powder is acid to litmus and has a faint odor of acetic acid. The pH of a 0.5 per cent solution was found to be 4.7. Free acid was determined by titration to phenolphthalein end-point. When distilled (3), acetic acid was identified as the only volatile acid present. Total titrations on the basis of 250 ml distilled were calculated to this acid. The following results were obtained:

	Per cent
Free acid calculated as acetic	34.02
Total volatile acids as acetic	76.81
Combined acid (By difference)	42.79
Combined acid (Calc. as anh. sodium acetate)	58.45
Total Calculated	92.47

The figure of 76.8 per cent total available acetic acid was used in calculating recoveries of added "sodium diacetate."

(3) *White flour—sodium propionate mixture.*

Added—0.20% sodium propionate

Determined:—Formic Acid— $\left. \begin{array}{l} 1.3 \\ 1.3 \end{array} \right\}$ Av. 1.3 mgs./100 gms.

Acetic Acid—None detected

Propionic Acid— $\left. \begin{array}{l} 168.0 \\ 165.0 \end{array} \right\}$ Av. 166.5 mgs./100 gms.

Calculated:—Sodium Propionate—216.5 mgs./100 gms. (0.22%)

(4) *White flour—sodium diacetate mixture.*

Added—0.25% sodium diacetate

Determined:—Formic Acid— $\left. \begin{array}{l} 1.3 \\ 1.5 \end{array} \right\}$ Av. 1.4 mgs./100 gms.

Acetic Acid— $\left. \begin{array}{l} 204.1 \\ 200.8 \end{array} \right\}$ Av. 202.5 mgs./100 gms.

Propionic Acid—None detected

Calculated:—Sodium Diacetate—263.7 mgs./100 gms. (0.26%)

(5) *Flour.*

(a) *White flour*. Previous analyses of starch and white flour by the volatile acid procedure (2) indicated trace amounts of acetic acid (less than 0.01%).

(b) *Whole wheat flour*. The whole wheat flour used in the preparation of the wheat bread was analyzed (2) with the following results:

Formic Acid	$\left. \begin{array}{l} 3.8 \\ 3.4 \end{array} \right\}$	Av. 3.6 mgs./100 gms.
Acetic Acid	$\left. \begin{array}{l} 7.0 \\ 7.5 \end{array} \right\}$	Av. 7.3 mgs./100 gms.
Propionic Acid		None

[The above described analyses show that the inhibitors were thoroly mixed with the white flour and that the method gave efficient recoveries when applied to these mixtures. Recovery experiments were previously reported wherein propionic acid was added to air dried bread (1)].

(6) *White and Whole Wheat Breads—Propionate and Diacetate Recovery.*

The results of analysis for volatile fatty acids on control breads and breads containing added inhibitors are listed in Table 1. All of the results on fresh bread and on air dried samples have been calculated to the original fresh basis—one hour after baking.

DISCUSSION

Substantial losses of both propionate and diacetate have been found to take place during baking and for the most part additional greater losses during the air drying of bread in sample preparation. Bread is slightly acid and it would be expected that losses of the volatile acids would occur at the relatively high temperature of baking. The time required for the air drying of bread to about 10 per cent moisture depends on the relative humidity and circulation of air. From two to four days are allowed at this location. Apparently there is a gradual loss of propionic and acetic acids during the period of drying. Greater air drying losses are observed in the wheat bread than in the white bread. The pH of both types of the samples analyzed is about the same and does not afford any basis for conclusion as to the reason for this difference. The difference may have been due to the longer period of drying for the wheat bread.

Where propionates are used in amounts considered general commercial practice (0.2–0.25% in the flour or 0.12–0.16% in the fresh bread) they may be detected in the analysis of air dried bread by the volatile acid procedure described in the previous report. However, the results of the present experiments indicate the fresh sample basis to be preferable even though it is necessary to air dry for reserve and subsequent analysis.

No propionic acid was calculated in the control samples or in samples with added diacetate.

All previous and present analyses made by the Associate Referee in the determination of volatile fatty acids in bread show that acetic acid is normal in bread and in amounts that must be taken into account in the detection of added acetic acid whether from vinegar or sodium diacetate.

ACKNOWLEDGMENT

The Associate Referee acknowledges with appreciation the assistance of V. E. Munsey (U. S. Food and Drug Administration, Washington, D. C.) in the preparation of the bread samples that are the basis of the present report.

It is recommended* that investigation of an alternative chromatographic technique (4) for the identification and estimation of volatile acids in bakery products be undertaken.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 57 (1949).

TABLE 1.—*Losses of sodium propionate or sodium diacetate in baking and air drying calculated basis volatile acid determinations*
Results (mgs/100 gms fresh basis)

BREAD PRODUCTS	VOLATILE FATTY ACIDS					
	FRESH BREAD			AIR DRIED BREAD		
	FORMIC	ACETIC	PROPIONIC	FORMIC	ACETIC	PROPIONIC
White Control	2.0	56		1.4	44	
White Control	1.8	59		—	—	
White Control (Av.)	1.9	58	None	1.4	44	None
White + Diacetate	2.6	164		2.8	122	
White + Diacetate	2.8	166		2.1	117	
White + Diacetate (Av.)	2.7	165	None	2.5	120	None
White + Propionate	2.6	59	80	2.4	36	68
White + Propionate	2.8	70	66	—	47	58
White + Propionate	—	61	81	—	—	—
White + Propionate	—	66	76	—	—	—
White + Propionate (Av.)	2.7	64	76	2.4	42	63
Whole Wheat Control	8.4	50		7.0	33	
Whole Wheat Control	—	—		—	35	
Whole Wheat Control (Av.)	8.4	50	None	7.0	34	None
Whole Wheat + Diacetate	9.2	158		10.3	100	
Whole Wheat + Diacetate	9.0	155		—	100	
Whole Wheat + Diacetate (Av.)	9.1	157	None	10.3	100	None
Whole Wheat + Propionate	9.0	44	101	8.8	32	70
Whole Wheat + Propionate	8.6	35	105	—	30	72
Whole Wheat + Propionate (Av.)	8.8	40	103	8.8	31	71

TABLE 1.—Continued

BREAD PRODUCTS	SODIUM PROPIONATE						SODIUM DIACETATE					
	ADDED	RECOVERED		LOSSES		TOTAL	ADDED	RECOVERED		LOSSES		TOTAL
		FRESH	AIR DRY	BAKING	AIR DRYING			FRESH	AIR DRY	BAKING	AIR DRYING	
				per cent	per cent	per cent				per cent	per cent	per cent
White Control	None	None	None	—	—	—	None	76 ¹	57 ¹	—	—	—
White Control (Av.)	None	None	None	—	—	—	157	215	156	8 ²	25 ²	33 ²
White+Diacetate												
White+Diacetate												
White+Diacetate (Av.)												
White+Propionate												
White+Propionate												
White+Propionate												
White+Propionate (Av.)	124	99	82	20	14	34	None	82 ¹	54 ¹	—	—	—
Whole Wheat Control												
Whole Wheat Control												
Whole Wheat Control (Av.)	None	None	None	—	—	—	None	65 ¹	44 ¹	—	—	—
Whole Wheat+Diacetate												
Whole Wheat+Diacetate												
Whole Wheat+Diacetate (Av.)	None	None	None	—	—	—	157	204	130	8 ²	33 ²	41 ²
Whole Wheat+Propionate												
Whole Wheat+Propionate												
Whole Wheat+Propionate (Av.)	155	134	92	14	27	41	None	52 ¹	40 ¹	—	—	—

¹ Normal acetic calculated to sodium diacetate.
² Per cent loss of total acetic acid (added plus normal).

REFERENCES

- (1) McROBERTS, L. H., *This Journal*, 31, 497 (1948).
- (2) *Changes in Methods of Analysis*, A.O.A.C., *Ibid.*, 31, 99 (1948).
- (3) *Methods of Analysis*, A.O.A.C., 6th Ed. (1945) (24.9-24.10).
- (4) RAMSEY, L. L., and PATTERSON, W. I., *This Journal*, 28, 644 (1945).

REPORT ON THIOUREA IN FOODS

By W. O. WINKLER (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In last year's report, two methods were proposed for the determination of thiourea; one for oranges or orange juice and the other for frozen dessert peaches. These methods were proposed as tentative. The one for orange juice employed amyl alcohol for the extraction of thiourea from the concentrated sample and the removal of the thiourea from the amyl alcohol by a water extraction. This procedure was followed in order to separate the thiourea from a substance in the juice which inhibited the reaction with a hydrated form of sodium cyano ferrate (modified Grote's reagent).

It was stated in last year's report that the inhibitor appeared to be a reducing agent. Experiments this year have shown that ascorbic acid exerts a similar inhibitory effect on the reaction between thiourea and the modified Grote's reagent. The ascorbic acid in the juice therefore is evidently one substance which inhibits the above reaction. Inasmuch as the reagent apparently oxidizes¹ the thiourea first to formamidine disulfide before the coupling reaction, it seems possible that the effect of the inhibitor (ascorbic acid) might be removed by a preliminary oxidation of the sample. At the same time, the thiourea (being easily oxidized) would be oxidized to the disulfide stage and could be determined if conditions were such that it remained stable during the course of the determination.

The disulfide has been shown to form rather stable salts with some strong acids. However, the reaction with the cyano ferrates does not take place readily in too strong acid solutions.

In view of these findings a direct and rapid method was devised which gave excellent results, in the hands of the Associate Referee, on samples of fresh orange juice containing known quantities of added thiourea. Briefly, the method consists of the clarification of the strained orange juice, oxidation with excess of standard iodine solution in strong acid, reduction of acidity with sodium acetate, and determination of thiourea by use of sodium cyano ferrate reagent (modified Grote's reagent) and a photometer. Some results obtained by the Associate Referee are given in Table 1.

In the opinion of the Associate Referee, the results obtained are excellent.

¹ *This Journal*, 31, 476 (1948); *Analyst*, 71, 562 (1946).

A sample of orange juice was prepared, to which was added 30 p.p.m. of thiourea, and after thorough mixing was placed in pint bottles having plastic caps. The bottles were loosely capped and placed in an oven at 100°C for 30 minutes to sterilize the sample. The bottles were then tightly capped and allowed to cool. After cooling and standing, the samples appeared dark, with a rather brownish cast.

One bottle of the sample was sent to each of a number of collaborators, with the request that they determine thiourea both by the tentative method proposed last year and by the new rapid oxidation method. The results obtained by collaborators and the Associate Referee were disap-

TABLE 1.—*Results of thiourea determination on fresh orange juice, by the rapid oxidation method*

THIOUREA ADDED	THIOUREA FOUND	DIFFERENCE
p.p.m.	p.p.m.	p.p.m.
9.1	9.2	+0.1
18.2	18.2	+0.0
30	29.0	-1.0
27.3	27.4	+0.1

pointing. Those obtained by the rapid oxidation method varied from 20.7 to 24.9 p.p.m., and results by the tentative method varied from 16.5 to 26.0 p.p.m.

Immediately after making the determination on the A.O.A.C. sample, the Associate Referee prepared a sample of fresh orange juice to which were added 30 p.p.m. of thiourea; no heat treatment was applied. Determinations of thiourea were made on this sample by the rapid oxidation method and recoveries of 29.0 and 29.1 p.p.m. were obtained.

It is the opinion of the Associate Referee, therefore, that the heat treatment for sterilization of the A.O.A.C. sample was too drastic and destroyed a portion of the added thiourea and partially caramelized some of the sugars, as shown by the dark color. No doubt this accounts for the low, rather erratic results obtained by collaborators. Comments by collaborators indicated that the rapid oxidation method was much preferred to the tentative method. The rapid method is proposed as a tentative method until further collaborative work can be done. Details of the rapid oxidation method are given in the February number of *This Journal*, on page 100, under "Changes in Methods of Analysis."

RECOMMENDATIONS*

It is recommended—

(1) That the rapid oxidation method for thiourea in oranges or orange

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 58 (1949).

juice, given in this report, be adopted as tentative, and that the present tentative method for thiourea in orange juice be dropped.

(2) That work on the detection and determination of thiourea in foods be continued.

REPORT ON FORMALDEHYDE

By HOWARD P. BENNETT (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

Methods for the detection of the toxic preservative, formaldehyde, have not been extensively studied by the Association for many years. Work done by Oakley (1) pointed up a need for restudy of this topic. Two recent references in the literature (2, 3) show that the use of this compound is not exactly a dead issue and there is an ever present temptation to apply it to food material.

The Association has indicated that present unused and out-dated methods should be deleted from the *Methods of Analysis* and newer methods for the detection of formaldehyde studied. The study of Oakley, mentioned above, shows well the strength and weakness of present methods. The writer undertook the study of some new methods; and on the basis of these studies, the following recommendations are made.

RECOMMENDATIONS*

It is recommended that the Hehner Test (32.21) for testing milk be studied as modified according to directions of Fulton (4), as follows:

HEHNER-FULTON TEST FOR FORMALDEHYDE IN MILK

Dilute 8 ml of concd. sulfuric acid with 5 ml of water, cool, and put 4 ml of this diluted acid in a test tube. Add 1 ml of the milk to the sulfuric acid slowly and mix with cooling. A clear and practically colorless soln results unless a large proportion of formaldehyde is present. Prepare a bromine oxidizing soln by mixing equal volumes of concd. sulfuric acid and saturated bromine water, and cooling. Add ca 0.5 ml of this oxidizing soln to the sulfuric acid milk soln and shake. In the presence of formaldehyde a violet color develops at once, a color ranging to light purplish pink for very small amounts of formaldehyde. Make a blank determination for comparison.

It is further recommended that, the two following methods be subjected to collaborative study at an early date.

TANNIC ACID TEST FOR FORMALDEHYDE

Reagent.—Dissolve 50 mg of tannic acid in 100 ml of concd. sulfuric acid.

THE TEST

To 5 ml of the reagent in a small casserole add 1 ml of the distillate (32.19). Heat on steam bath for 5 min. The appearance of a green or blue-green color indi-

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 57 (1949).

ates formaldehyde. Make a blank determination for comparison. (As little as 2 micrograms of formaldehyde can be detected.)

CHROMOTROPIC ACID TEST FOR FORMALDEHYDE (5)

Reagent.—Make a saturated soln of purified chromotropic acid (ca 500 mg/100 ml) in ca 72% sulfuric acid.

THE TEST

Place 5 ml of the reagent in a test tube, and add with mixing 1 ml of the distillate (32.19). Place in a boiling water bath for 15 min. The presence of formaldehyde is indicated by a light to deep purple color, depending upon the amount present. (As little as 4 micrograms may be detected, and the test is very specific.)

LITERATURE CITED

- (1) OAKLEY, MARGARETHE, *This Journal*, **28**, 296 (1945).
- (2) *Chemical Abstracts*, **38**, 4706 (1944).
- (3) *Ibid.*, **42**, 2687 (1948).
- (4) FULTON, C. C., *Ind. Eng. Chem., Anal. Ed.*, **3**, 199 (1931).
- (5) EEGRIWE, EDWIN, *Z. Anal. Chem.*, **110**, 22 (1937).

No report was given on saccharin, dichloroacetic acid, dulcin, or 1-propoxy, 2-amino, 4-nitro benzene.

No report was given on fill of container methods (food, drugs, and cosmetics), or on coffee and tea.

REPORT ON EGGS AND EGG PRODUCTS

By FRANKLIN J. McNALL (Food and Drug Administration) Federal Security Agency, Cincinnati, Ohio), *Referee*

ADDED GLYCEROL

During the past year the Associate Referee, George Keppel, has devised a periodate method for the determination of added glycerol. It is recommended that this work be continued and the method be submitted for collaborative study.

ACIDITY OF FAT

No report on acidity of fat was given by the Associate Referee. It is recommended that this study be continued.

RECOMMENDATIONS*

In line with the Report of Committee on Classification of Methods, the following recommendations are made:

(1) That an Associate Referee be appointed to study the present tentative method for Fat by Acid Hydrolysis (23.8). This method was adopted

* For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 53 (1949).

as tentative in 1933 but was never made official because of the lack of collaborative study.

(2) That the tentative Qualitative Method for Glycerol (23.25) be made official, first action. This test was given some collaborative study in 1932 and 1933 and it should be made official.

(3) That the tentative Rapid Method for Acidity of Ether Extract (23.31) be made official, first action. The 1938 report shows method gives results in close agreement to present official method (23.29).

(4) That an Associate Referee be appointed to study the tentative Method for Ammonia Nitrogen (23.33). It is our understanding that several states use this method or a similar one for the interpretation of cases involving the age of shell eggs.

(5) That an Associate Referee be appointed for Succinic Acid in Eggs and that the method be studied collaboratively next year.

REPORT ON ADDED GLYCEROL IN EGGS AND EGG PRODUCTS

By GEORGE E. KEPPEL (Food and Drug Administration, Federal
Security Agency, Minneapolis 1, Minn.), *Associate Referee*

Studies were continued with special reference to the problem of isolating glycerol from interfering compounds normally occurring in egg yolk and in whole egg. The method used for examining the isolated glycerol is based on a modification of the periodate oxidation procedure by Newburger and Bruening.¹ To obtain a sharper end point in titrating the formic acid formed, the excess periodate is reduced, and bromcresol purple is substituted for methyl red as the indicator.

EXPERIMENTAL

Samples of fresh egg yolk containing no added glycerol were mixed with water and clarified by treatment with sodium tungstate solution and dilute sulfuric acid. This is essentially the method of Folin and Wu, commonly used in blood analysis for the preparation of protein-free blood filtrate. Aliquots of the egg filtrate, analyzed by the modified periodate method, show blanks of 0.2–0.3 per cent apparent glycerol. A number of other clarification reagents gave poorer results. Lead acetate, for example, introduces a buffer effect that interferes with the subsequent neutralization and titration. Lead nitrate-sodium hydroxide gives excellent clarification but the excess lead reacts with periodate and must be removed by separate steps. The same is true of other precipitants such as copper, zinc, or

¹ Newburger, S. H., and Bruening, C. F., *This Journal*, 30, 651 (1947).

mercury salts. Phosphotungstic acid yields clear filtrates, but titration end points are not as sharp as with tungstate.

The nature of the interfering substances is not definitely known, but it appears that it may consist chiefly of dextrose, small amounts of which are normally present in eggs. Dextrose is one substance which would not be removed by the above clarification methods and which will yield acid by periodate oxidation.

As the result of a number of experiments designed to eliminate or reduce dextrose interference, a technique was devised which is effective in reducing blank values to the range of 0.05–0.08% apparent glycerol. An aliquot of the filtrate is made alkaline and heated to boiling. This treatment

TABLE 1.—*Effect of alkaline treatment on dextrose*

DEXTROSE	APPARENT GLYCEROL	APPARENT GLYCEROL AFTER ALKALINE TREATMENT	REDUCTION IN APPARENT GLYCEROL
mg	mg	mg	per cent
5	3.96	1.34	66.2
10	7.92	2.78	64.9
25	19.80	7.80	60.8
50	39.6	15.07	61.9
100	79.2	25.30	68.1

is based on the instability of reducing sugar solutions when heated in the presence of alkali. The solutions become yellow to brown in color, and various products of an acid nature are among the substances formed. According to Browne and Zerban² lactic acid is produced in considerable amount by the action of alkalies upon many reducing sugars in the presence of air or oxidizing agents. Among the other oxidation products, formic acetic and oxalic acid have been found.

Results on dilute dextrose solutions treated with alkali and heated are shown in Table 1. In each case the dextrose solution was diluted to 25 ml. in an Erlenmeyer flask, 2 ml. of 10% NaOH added, mixed, heated to boiling, and boiled 15 seconds. To eliminate carbonate which interferes with the formic acid titration, the alkaline mixture is acidified, again boiled and cooled. It is then neutralized and the glycerol determination made.

The results show that not all of the dextrose interference can be removed by alkaline treatment. However, in the case of quantities of sugar comparable to those found in normal eggs, the interference can be reduced to a relatively small value.

On the basis of the experimental work described, a tentative method was devised to be used on egg products containing no added sugars.

² Browne, C. A., and Zerban, F. W., "Physical and Chemical Methods of Sugar Analysis," p. 658, Third Edition (1941).

METHOD

REAGENTS

Sodium tungstate soln.—Dissolve 10 g of reagent grade sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) in water and dilute to 100 ml.

Potassium periodate, .02 M.—Dissolve 4.6 g of C.P. KIO_4 in ca 500 ml of hot water. Dilute to about 900 ml with water, cool to room temp., and make to 1 liter.

Sodium hydroxide, .02 N.—Dilute 100 ml 0.1 N NaOH to 500 ml with CO_2 -free water.

Bromcresol purple indicator.—Dissolve 0.1 g of indicator in 100 ml of alcohol.

Propylene glycol.—Use a pure product containing no free acidity or liberating acidic substances on oxidation.

DETERMINATION

Weigh accurately by difference ca 2 g of well mixed sample 23.1(a) or (b) into 100 ml volumetric flask containing 50–75 ml of H_2O . Mix and add 2 ml of the sodium tungstate soln. Add slowly with continuous mixing 2 ml of 1 N H_2SO_4 . Dilute to mark with H_2O , mix well, and filter (18.5 cm folded filter).

Transfer 20 ml of filtrate containing not more than 40 mg of glycerol to a 300 ml Erlenmeyer flask. Add 2 ml of 10% NaOH, heat to boiling, and boil for 30 sec. Cool slightly, add 2 drops of bromcresol purple indicator, neutralize with 1 N H_2SO_4 and add 2–3 drops excess. Boil 1 min. and cool to room temp. Neutralize with 0.02 N NaOH, titrating to light purple shade.

Add ml 0.02 M KIO_4 , mix, wash down sides of flask with H_2O , and allow to stand 30 min. Add 10 drops of propylene glycol to the oxidized mixture, mix and let stand for 10 min. Add 3 drops of bromcresol purple indicator and titrate with 0.02 N NaOH.

1 ml 0.02 N NaOH = 1.84 mg. glycerol.

To correct for error due to volume occupied by insoluble matter, repeat determination, weighing same quantity of sample into 200 ml volumetric flask, and proceed as directed above, except that a 40 ml aliquot is used for the determination instead of 20 ml. To obtain amount of glycerol subtract % glycerol obtained in 100 ml dilution from twice % obtained in 200 ml dilution determination.

Results on a variety of egg products containing no added glycerol are shown in Table 2, indicating the blank range and substances which interfere.

TABLE 2.—Results on egg products containing no added glycerol

PRODUCT	APPARENT GLYCEROL
	<i>per cent</i>
Whole egg	0.05
Egg yolk	0.05
Egg white	0.08
Frozen whole egg (decomposed)	0.07
Dried whole egg	0.06
Whole egg containing 10% salt	0.06
Whole egg containing 10% sucrose	0.63
Whole egg containing 3.53% dextrose	0.67

As shown, sucrose and dextrose interfere appreciably. Salt has no apparent effect. The frozen whole egg sample was a commercial product classified as decomposed when originally sampled. After sampling, it had been kept under cold storage for about 1 year. The product had a strong disagreeable acid odor.

Results on recovery of glycerol added to whole egg or egg yolk are tabulated under Table 3. Glycerol used was USP grade glycerin, and its strength was checked by periodate oxidation, by dichromate oxidation, and by specific gravity. The various batches were prepared by weighing glycerol and fresh egg, laboratory separated, into glass-stoppered flasks. Contents were mixed by shaking the closed flask and allowed to stand overnight in a refrigerator to insure uniformity.

TABLE 3.—*Application of method to eggs containing known amounts of glycerol*

PRODUCT	GLYCEROL		RECOVERY
	PRESENT	FOUND	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Egg yolk	9.74	9.85	101.1
		9.91	101.7
Egg yolk	11.44	11.56	101.0
		11.60	101.4
Egg yolk	7.36	7.44	101.1
		7.42	100.8
Whole egg	10.40	10.41	100.1
		10.35	99.5
		10.39	99.9

These results are uncorrected for volume occupied by insoluble material. Table 4 gives results on a series corrected by the method of double dilution and a comparison with the uncorrected results.

TABLE 4.—*Glycerol results of double dilution method*

PRODUCT	GLYCEROL PRESENT	GLYCEROL FOUND		RECOVERIES	
		UNCORRECTED	BY DOUBLE DILUTION	UNCORRECTED	DOUBLE DILUTION
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Egg yolk	7.36	7.43	7.38	101.0	100.3
Whole egg	7.68	7.71	7.69	100.4	100.1
Whole egg	10.40	10.38	10.34	99.8	99.4

An unsuccessful attempt was made to obtain samples of commercial

egg preparations containing added glycerol for examination by the above method. It appears that very little of this type product is being manufactured at the present time because of the current high price of glycerol.

Because of lack of time no comparisons have been made with the present tentative method for glycerol, and no collaborative work has been done.

It is recommended* that study on methods for isolating glycerol from egg mixtures be continued.

No report was given for acidity of fat (in egg products).

A contributed paper, by F. Hillig, on "Water-insoluble Acids in Dried Eggs," was published in *This Journal*, 31, 731 (1948).

No report was given on microbiological methods for canned fishery products, canned meats, canned acid foods, canned vegetables, eggs and egg products, nuts and nut products, frozen fruits and vegetables, and sugar.

REPORT ON DECOMPOSITION IN FOODS

By W. I. PATTERSON (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

RECOMMENDATIONS†

Recent progress in the chemical detection of cow manure in milk suggests that the subject of filth in foods might be included under a title still to be selected in the 1949 A.O.A.C. list of subjects. In the Referee's opinion, the simplest way is to combine it with decomposition, one possible title being "Chemical Indices of Filth and Decomposition in Foods." It is recommended that the scope of the subject "Decomposition in Foods" be broadened to include chemical methods for detecting filth in foods.

The Referee concurs in the recommendations of the Associate Referees for decomposition in foods, as follows:

(1) For decomposition in dairy products, that the method for water insoluble acids in butter and cream be adopted as official first action, and that the study of other chemical methods for decomposition in dairy products be conducted.

(2) For decomposition in fish, that the revised method for volatile acids in fish be studied collaboratively.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 53 (1949).

† For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

(3) For decomposition in fruit and fruit products, that the study of chemical tests for "blackheart" in pineapple be developed further, and that the study of other possible chemical means of detecting decomposition in fruit products be pursued.

REPORT ON DECOMPOSITION IN FRUITS AND FRUIT PRODUCTS

By W. O. WINKLER (Food and Drug Administration, Federal Security Agency, Washington D. C.), *Associate Referee*

INTRODUCTION

The first report on decomposition in fruit products was made last year by T. H. Harris.¹ That report dealt mainly with the problem of detecting the use of rotten fruit in the preparation of apple juice or apple butter. After considerable research, three products of decomposition, probably from the pectin, were found. These were an alcohol soluble non-dialyzable substance, alcohol soluble furfural-yielding substances, probably pentoses, and D galacturonic acid. Of these products Dr. Harris concluded that the D galacturonic acid offered more promise as a criteria of rot than the other substances studied. A method was proposed for the determination of D galacturonic acid, which was a modification of the Deichman and Dierker method² using naphthoresorcinol. It was shown by the method that some of the galacturonic acid was present in sound, ripe fruit, but the amount in rotten fruit greatly exceeded (20 times or more) that in the sound fruit. However, the reagent used is not specific for galacturonic acid, producing small quantities of color with various other acids. A study of the conditions of the reaction may further improve the specificity of the reagent.

Determinations of galacturonic acid in sound and rotten apples have been made by the writer, using the proposed method, but the results showed somewhat less difference in galacturonic acid content of these substances than was reported by Dr. Harris. A part of this reduction in the ratio of rotten to sound fruit may be due to the greater age of the materials used. Further study of the reaction conditions should render the method more valuable.

The writer has approached the problem more from the biochemical standpoint than as a strictly organic chemistry problem. Nearly all food decomposition is the result of the biological action of microorganisms, the chemical changes which they initiate being the result of the action of enzymes which these organisms secrete. The problem is therefore closely associated with the metabolism of the various microorganisms. The products resulting from the metabolic action are different in different groups

¹ *This Journal*, 31, 501 (1948).

² *J. Biol. Chem.*, 163, 753 (1946).

but probably similar in related species. The decomposition of most fruits is brought about by the action of molds or yeasts. Bacterial action causing rot in fruit is rather rare.

Many fruits and, no doubt, other foods are attacked generally by a particular group of microorganisms. Rot in apples is usually caused by the molds *Penicillia expansum* or the *Mucors*, particularly *Mucor racemosus*. In oranges or citrus fruits we usually have *P. digelatum* and *P. italicum*.³ *Rhizopus nigricans* is important in the spoilage of strawberries and stored potatoes,³ etc.

The biochemistry of the molds has been studied systematically and very extensively by Raistrick⁴ and his co-workers in England. These investigators have cultured many of the fungi, have made an intensive study of the media, and have isolated many of the products of mold metabolism. They have isolated and studied numerous compounds resulting from the biological processes of the various species of these organisms. They developed a rather ingenious scheme of analysis for determining the presence of metabolic products in the culture filtrate. The writer believes it would be profitable to follow a course similar to the one they have outlined.

Among the rather common compounds which have been isolated as products of mold metabolism are: ethyl alcohol, acetic acid, formic acid, oxalic acid, citric acid, succinic acid, fumaric acid, mannitol, butanol acetaldehyde, glycerol, acetone, fatty acids, and galacturonic acid. Among the products which are more particularly characteristic of mold metabolism are kojic, furoic, aspergillic, penicillic, carolic, carolinic, carlic, carlosic, aconitic, gentisic, fulvic, luteic, glycolic, and tetrionic acids; citrinin, citromycetin, holetol, catenarin, and other anthroquinone derivatives; sterols, glycerides, and some polysaccharides. Ethyl acetate is also a rare product of some molds. Numerous other substances have also been found as products of mold metabolism.

The compounds citrinin and citromycetin are produced by the molds *Penicillium citrinum* and *Citromyces* and appear to be characteristic of these organisms. The determination of these constituents would be effective in establishing the use of moldy, decomposed fruit.

Time did not permit of a search for many of the products enumerated, but a limited search was made for the presence of mannitol, kojic acid, glycerides, sterols, and polysaccharides in rotten and sound apples. The presence of mannitol was investigated by two polarimetric methods based on rotation as a result of complex formation of the mannitol with borax or molybdate. These methods gave indication of the presence of mannitol in rotten apples in significant amounts, but also showed lesser and vary-

³ *J. Soc. Chem. Ind., London*, 581 (1936).

⁴ *Trans. Roy. Soc. (London)*, B 220, 1-387 (1931), and *Biochem. J.*, 26, 1441 (1902), 1907 (1932), to 1947.

ing amounts in different varieties of sound apples. It is thought probable that other substances may affect the determination, and more sensitive and specific methods for mannitol will be sought.

Tests for kojic acid, in the rotten apple extracts tested, proved negative.

Tests on glycerides and sterols were not conclusive, an account of the difficulty of removing the natural waxes; but indications of the presence of sterols were obtained and these substances will be further investigated.

A study of the constituents of rotten apple tissue revealed the presence of a substance which appears to be a polysaccharide but not a starch or a pentosan. The material is of a mucilaginous character and was present in considerable quantity, approaching 0.8%, in rotten apple juice, but in very small quantity in the sound apple juice. The identity of this material has not been determined but will be investigated, and its use as a criterion of rot, particularly in apples, will be ascertained.

"BLACKHEART" IN PINEAPPLE

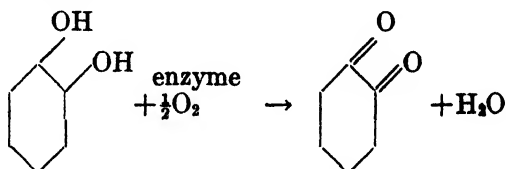
At the beginning of the work on decomposition of fruits, the problem of detecting the condition called "blackheart" in pineapple which had been frozen in the fresh condition was assigned to the writer. There appears to be some confusion in the minds of the public regarding the terms "blackheart," "black rot," etc. As far as can be determined by the writer, the condition called "black rot" is due to the fungus *Thielaviopsis paradoxa*. This organism soon disintegrates the fruit to a watery consistency. The macrospores of the fungus form on exposed tissues and on decayed tissues near the core of the fruit, turning it black.⁵

In the condition known as "blackheart" and also termed "internal breakdown," fungal growth or spores are not apparent. The flesh becomes dark near the core and spreads outward toward the surface. The discolored flesh often appears firm but is flat and insipid to the taste. Examination of a considerable number of these fruits (containing so-called "blackheart"), by the Microbiological Division of the Food and Drug Administration, failed to reveal the presence of the spores or hyphae of fungus growth. Also, samples sent to the Agricultural Research Center at Beltsville, Maryland, failed to disclose the presence of microorganisms. The decay or breakdown appears to be brought about as the result of an abnormal physiological development and is stimulated or accelerated by adverse conditions of moisture and temperature. The problem was attacked from this point of view. In cooperation with W. I. Patterson, the Referee, it was determined that the darkened color in the fruit was no doubt a melanin type compound probably resulting from the enzymatic oxidation of the amino acid tyrosine.

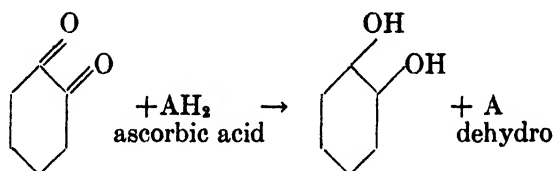
A search of the literature was necessary to obtain a satisfactory method for tyrosinase (catecholase) activity. The method finally used is a modifi-

⁵ U.S.D.A. Circular No 511.

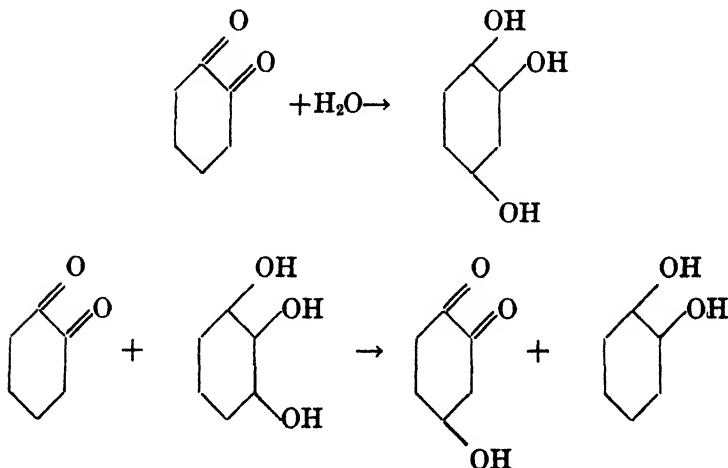
cation of the chromometric method of Dawson and Nelson,⁶ and is based on the catecholase activity of the material. Results by the method showed a very striking difference between the sound and the blackheart samples. Of the samples so far tested, blackheart samples showed comparatively strong catecholase activity, while sound, just-ripe samples of pineapple showed only a trace or no activity. Sound, rather overripe samples showed only a weak activity. Although the catecholase activity test has not been proven specific for blackheart, it has been found to be capable of detecting the presence of blackheart in the authentic samples examined. Therefore, the procedure is proposed as a method for the detection of blackheart or of other types of decomposition in samples of fresh frozen pineapple which may show catecholase activity. The reactions involved in the determination are as follows:



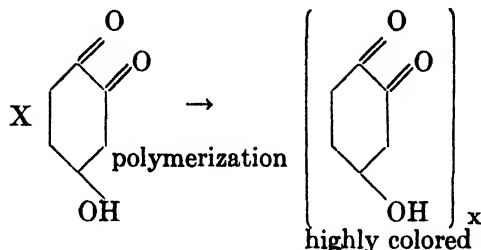
When ascorbic acid is present:



When no ascorbic acid is present or it has been used up, the reaction of the o-benzoquinone continues as follows to form the colored product.



⁶ J. Amer. Chem. Soc., 63, 3375 (1941).



METHOD FOR THE DETECTION OF BLACKHEART IN FROZEN PINEAPPLE

REAGENTS

- (a) *Buffer soln.*—0.2 *M* citric acid.—0.4 *M* secondary sodium phosphate.
- (b) *Pyrocatechol soln.*—0.2%.—200 mg catechol in 100 ml.
- (c) *Sulfuric acid pyrogallol.*—2 *M* sulfuric acid containing 1% pyrogallol prepared fresh each day.
- (d) *Potassium iodide.*—10% soln of KI freshly prepared each day.
- (e) *Starch indicator*—1% soln of soluble starch.
- (f) *Ascorbic acid soln stock (strong).* 100 mg ascorbic acid U.S.P. in 100 ml of 0.1% metaphosphoric acid.—Keep at 40°F.
- (g) *Ascorbic acid (weak).* 10 mg per 100 ml.—Place 10 ml of soln (f) (stock) in a 100 ml flask and make to volume with 0.1 per cent metaphosphoric acid (HPO₃). Prepare fresh from reagent (f) each day.

PREPARATION OF SAMPLE

Cut the frozen material in the opened package with a knife completely across from side to side, cutting first in half, then in quarters, eighths, sixteenths, etc., until the contents are well chopped. Pour the chopped material into a large beaker or casserole, chop up any remaining lumps, and mix by stirring well.

Weigh 100 grams of chopped sample into a tared beaker (400–600 ml). Then add 100–110 grams of acetone and stir with a glass rod to mix contents. Decant mixture into a Waring blender and rinse in any material in the beaker with 60% acetone (volume). Thoroughly disintegrate the material by blending for 1 min.

Pour out the blended mixture into original beaker, allow to drain ca 20–30 seconds, and rinse blender well with 60% acetone. Filter mixture on a rapid fluted filter (E & D No. 195 is suitable) 18½ to 24 cm in diam. Allow the filtrate to run thru until there is only a slow dropping, then rinse the beaker and filter with ca 100 ml of 60% acetone. When almost all the liquid has passed thru and there is only a slow drip of filtrate, remove filter and precipitate to a wide-mouthed short-stemmed funnel set in an 800 ml beaker. Puncture filter and wash precipitate from the filter into the beaker with 60% acetone from a wash bottle. Stir to disperse the solid. Filter mixture on a Büchner funnel (11 cm. diam.) using two No. 54 or 41 H Whatman filter papers with aid of *gentle* suction. Rinse beaker and transfer any residue quantitatively to funnel with 60% acetone. When most of liquid has passed thru and residue has formed a rather firm cake on the filter (filtrate is only a trickle or dropping), wash residue with another 75–80 ml of the 60% acetone wash. Allow the residue to suck dry until no more filtrate passes thru the filter and the residue becomes a firm hard cake. Discard the filtrate.

Strip the filter from cake of pineapple residue and transfer the latter quantitatively to a dry Waring blender. Remove any solid from sides of funnel with a spatula. Add 195 ml (graduate) of water to the blender and blend for 1 min. Pour the blended material into a 250-ml centrifuge bottle, stopper and shake for 30 seconds,

then centrifuge for 12–14 min. at ca 1800 r.p.m. Remove the bottle and filter the supernatant liquid thru a rapid filter (E and D No. 195 is suitable). Use portions of filtrate (X) for determination of catecholase activity.

DETERMINATION OF CATECHOLASE (TYROSINASE) ACTIVITY

(A) *By oxidation of ascorbic acid—*

Place a 300–500 ml round-bottomed three-neck flask in a bath held at 25°C. $\pm 0.5^\circ$. Insert a glass tube having a spray bulb at end, fitted in a stopper in the one side-neck so that the tube extends to near bottom of flask. (This tube is to carry a current of air of ca 0.35 liter per min. from an air hose.) In the other side-neck, fit a capillary (1 mm bore) siphon tube the inside end of which will extend about two-thirds of the way below the surface of a quantity of 100 ml of liquid in the flask. The outside end of the tube is longer, extending below bottom of flask. Below the end of this tube is placed a 250-ml white casserole as a receiver.

Place 10 ml of buffer soln (a) in the three-neck flask. In each of two 250 ml white porcelain casseroles place 25 ml of reagent (c), 25 ml of reagent (d), and 5 ml of reagent (e), and stir with glass rods to mix. Place one of the casseroles as a receiver under siphon tube. (Measuring of these reagents into casseroles should be done immediately before time to add other reagents to flask.)

Measure 30 ml of the sample filtrate (X, above) into a graduate and 50 ml of water into a second (50 ml) graduate, and measure also 10 ml of reagent (b), catechol, into a 10 ml graduate. Pour ca 25 ml of the water from graduate into flask, then pour in the 30 ml of sample soln. Now add 1 ml reagent (g) (0.1 mg ascorbic acid*). Rinse the cylinder which contained the sample soln with remaining water in the graduate and pour into flask, rinsing down the sides of the latter. Rotate to mix contents, place in position, and immediately start the air bubbling thru the spray tube. (The rate should have been previously adjusted to 1 liter every 3 min.) Finally, pour in the 10 ml of catechol soln (b) and start a stopwatch when this is added. Rotate the flask once or twice to bring all of the last soln into the body of the liquid.

Now (within 15 seconds after addition of catechol) start the siphon by momentarily closing the center neck of the flask with a stopper. Gently stir the liquid in the receiver as the liquid drops in from the siphon tube. Note the time required to develop a pronounced pink color, and the time to produce a purple color in the receiving soln. Compare the color in the receiver with the liquid in the other casserole. A definite or pronounced pink color should not develop in less than 5* min. with sound pineapple samples, nor a purple color in less than 8* min. If a sample gives an almost immediate pink or purple color at the entrance of the drops, the activity can be more closely measured by using a larger quantity (0.4–0.5 mg) of ascorbic acid on another portion of sample soln (X). A strong catecholase activity indicates blackheart.

(B) *By the development of color in the aerated solution—*

Place 10 ml of buffer soln (a) in a 300 ml round-bottomed flask. Measure 30 ml of the prepared sample soln (X) into a graduate and 50 ml of water into a second graduate. Add a portion of the water to the flask, pour in the sample soln (X) and rinse the graduate with the remaining water. Finally, add 10 ml of reagent (b) (catechol). Rotate the flask a few times to mix the contents. Aerate by passing a stream of air thru the spray tube as in determination (A), at the rate of ca 0.35 liter per min. for a period of one hour. Remove the air tube, pour the soln into an Erlenmeyer flask, and call this soln "P" (phenol catechol treated).

Determine the increase of color and turbidity in the treated aerated soln P (phenol treated) over that of the original sample by obtaining the following color and turbidity index:

* These figures are subject to revision.

$$I_{+t} = (1 - T_{gp})$$

where

I_{+t} = color and turbidity index.

T_{gp} = transmittancy in the green (520 $M\mu$) of soln P compared to soln S

where

S is given a transmittancy of 1.

Dilute some of the original sample soln (X) with the same relative amounts of buffer and water as used in the aerated catechol-treated portion, but omit the addition of the catechol soln and substitute water in its place. Designate this prepared standard soln as "S." In a photoelectric photometer or spectrophotometer cell, place some prepared soln S, above, and using a wave length of 520 millimicrons set the instrument so that a reading of 100 (100% transmission) is obtained with this soln. In a second matched cell place some of the soln P and obtain the per cent transmission of this soln.

Obtain the color and turbidity index by the formula above. Repeat the readings after the solns "P" and "S" have stood overnight.

The index I_{+t} on sound samples of frozen pineapple should not be above 0.1* for the immediate reading, or more than 0.2* for readings after standing overnight.

Increase in color may be determined and referred to standard soln of caramel in glycerol if this is found more desirable.

DISCUSSION

Since the oxidation of a known quantity of ascorbic acid is used in one part of the method as a measure of the o-benzoquinone produced by the enzyme, it was reasoned that, if an analogous reaction occurred with the tyrosine in the sample, the ascorbic acid would be destroyed in a similar manner and no melanin compounds would be formed until all the ascorbic acid had been oxidized. Determinations of ascorbic acid, together with catecholase activity, were therefore made on samples of sound, fully ripe, just-ripe, or slightly underripe pineapple, and on samples of pineapple containing blackheart. Results of the determination of catecholase activity and ascorbic acid are given in Table 1.

Examination of the data show that no ascorbic acid remained in the samples of blackheart pineapple, and as previously stated, these samples gave relatively strong tyrosinase activity as measured by oxidation of catechol. Samples containing 80 or 90 per cent of sound pineapple and 20 or 10 per cent of blackheart pineapple, respectively, showed a rather readily distinguishable increase in tyrosinase activity.

RECOMMENDATIONS*

It is recommended—

- (1) That work on the detection of decomposition in fruits be continued.
- (2) That the study of the method for galacturonic acid as a criterion of rot in apple products be continued.
- (3) That the study of the polysaccharide, described in this report as a criterion of rot by mold, be continued.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

TABLE 1.—Analysis of various types of pineapple samples for tyrosinase activity and some chemical constituents

SAMPLE NO.	DESCRIPTION	TYROSINASE ACTIVITY			VITAMIN C mg/100 gm	ACIDITY AS CITRIC per cent	SUGAR AS INVERT AFTER INVERT per cent	SUGARS AS INVERT BEFORE INVERT per cent	MULLORS REACTION FOR TYROSINE
		A ¹ ASCORBIC ACID OXIDATION	CDs COLOR DEVELOPED OVERNIGHT BY OXIDATION OF CATECHOL	I ₄ +t					
5048 7A	Sound, fully ripe crushed pineapple	0.1 mg Asc pk in 6½ min.	± Grey	.195					
Sub A4	Sound, fully ripe crushed pineapple	0.2 mg Asc no purp	+ Yel Br		3.9	.55	13.95		
Sub A2	Sound, fully ripe crushed pineapple	0.1 mg Asc no pk in 6 min. light pk in 8½ min.	+ Yel						positive
Sub A5	Sound, fully ripe crushed pineapple	0.2 mg Asc pk in 4½ min. no purp	+ Br Yel		2.8	.49	12.2		
Sub B1	Sound, fully ripe crushed pineapple, with 1 part added sugars to 5 parts fruit	0.14 mg Asc pk in 6 min. no purp	+ Yel		3.7	.49	33.76	9.06	positive
Sub B2	Sound, fully ripe crushed pineapple, with 1 part added sugars to 5 parts fruit	0.1 mg Asc no color in indicator	+ Yel or Br Yel		2.2				
Sub C3	Sound, barely ripe, or slightly underripe crushed pineapple	0.15 mg Asc no color	Nil same as blank with no catechol		8.5	.76	7.98		positive
Sub C4	Sound, barely ripe, or slightly underripe crushed pineapple	0.1 mg Asc slight pk			9.2	.96		7.13	

Sub C5	Sound, barely ripe, or slightly underripe crushed pineapple		.088	7.6	.65	28.52	positive
Sub D	Sound, barely ripe or slightly underripe crushed pineapple with 1 part sugar to 5 parts fruit	0.2 mg Asc very faint color in 10 min.	No developed color				
Sub H1	Straight crushed pineapple containing blackheart	0.4 mg Asc purp in 4½ min.	+++ Black	nil			
Sub H2	Straight crushed pineapple containing blackheart		+++	nil			
Sub H4	Straight crushed pineapple containing blackheart	0.2 mg Asc pk in 50 sec. purp in 3¼ min.	Br Bk +++ Dk Br to Black	nil	.46	9.8	
Sub H5	Straight crushed pineapple containing blackheart	0.4 mg Asc purp in 4 min. 40 sec.	+++ Black	nil	.43	8.8	8.9
Sub K6	Ground pineapple containing 10% blackheart		± Light Br	8.0	87		positive
Sub L1	Ground pineapple containing 22% blackheart by weight	0.1 mg Asc purp in 5 min.	++ Brown				
Sub L2	Ground pineapple containing 22% blackheart by weight		+++ Dk Br	3 6			

¹ The method used was essentially that of Miller and Dawson (*J. Amer. Chem. Soc.*, 63, 3375 (1941)), but using lower quantities of ascorbic acid (0.1 to 0.4), as described herein. After the addition of the catechol the time of aeration was measured with a stopwatch to the appearance of a pink or purple color. After several minutes a pink color was found to gradually develop in the indicator solution even though very little or no catecholase activity was present. After a few determinations, this difficulty was overcome by preparing reagents fresh each day.

Abbreviations used are: Ascorbic acid = Asc; pk for pink and purp for purple.
² Most of the determinations recorded here were made before it was decided to measure the "darkening" quantitatively as proposed in the method (b) herein. After a time the color intensity (darkening) was estimated visually and indicated by the following signs: "++" showed some or a small amount of color, and "+++," etc., indicated increasing color intensity or darkening.
 Abbreviations used in column, Dk = dark; Br, brown; Bk, black.

(4) That the determination of mannitol as an index of mold decomposition in fruits be continued.

(5) That other compounds elaborated by microorganisms, as indicated in this report, be investigated.

(6) That the method for detection of blackheart in pineapple by tyrosinase activity, given in this report, be studied further.

REPORT ON DECOMPOSITION IN DAIRY PRODUCTS

WATER-INSOLUBLE FATTY ACIDS IN CREAM AND BUTTER

By FRED HILLIG (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

In a previous paper¹ a method for the determination of water-insoluble fatty acids in cream and butter was proposed. The method was submitted to the New Orleans, Cincinnati, and St. Louis Stations of the Food and

TABLE 1.—WIA in butter—collaborative results

COLLABORATOR	W.I.A.	COLLABORATOR	W.I.A.
	<i>mg/100 grams</i>		<i>mg/100 grams</i>
	<i>Cincinnati Station</i>		<i>St. Louis Station</i>
	796	4	2484
1	762		2574
	773	5	2640
	763		2668
	843		<i>New Orleans Station</i>
2	790		<i>Sample 1</i>
	852	6	356
	862		343
	830	7	354
3	788		376
	783		<i>Sample 2</i>
	813	6	344
			349
		7	364
			334

Drug Administration for trial. The results obtained by these stations on samples of butter obtained in their respective cities are given in Table 1.

¹ *This Journal*, 30, 875 (1947).

Duplicate determinations were satisfactory and the analysts at the respective stations closely checked each other. No comparison of results between stations is possible, since each station had different butter samples on which to work.

Four samples of butter were next submitted to collaborators in Cincinnati, St. Louis, New Orleans, and Washington. The results given in Table 2 are satisfactory.

TABLE 2.—*WIA in butter—all collaborative results: same sample*
mg/100 g

COLLABORATOR	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
3	178	245	248	420
	176	240	242	437
2	168	232	250	432
	165	224	250	447
4	161	207	180	421
	164	200	178	413
8	163	200	237	429
	159	210	247	432
9	158	218	250	428
	159	212	249	424
10	157		195	424
	165	268	186	432

It is recommended that the method for the determination of water-insoluble fatty acids in cream and butter be adopted as official first action. It is further recommended that the study of chemical methods for the detection of decomposition in dairy products be continued.

The Associate Referee wishes to thank the following members of the Food and Drug Administration who collaborated in this work: F. M. Garfield and M. A. Braun, St. Louis Station; S. D. Fine, F. J. McNall, and H. C. Van Dame, Cincinnati Station; George McClellan, R. E. Duggan, and H. P. Bennett, New Orleans Station; and Dorothy Montgomery, Food Division, Washington, D. C.

REPORT ON DECOMPOSITION IN FISH PRODUCTS

By FRED HILLIG (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Subsequent to the work which led to the adoption of the method for volatile acids as official,¹ a method appeared in which a mixture of volatile acids was separated on a chromatographic column and the amount of each acid from the column determined by titration.² Numerous analyses on fish have shown this procedure to be satisfactory, once the volatile acids are isolated from the fish. This is accomplished by a simplified steam distillation in which the carefully controlled conditions of the former method are unnecessary. The equations used in the former method for calculating the results are not needed in the chromatographic procedure, although a simple calculation based on the percentage of each acid which distills in 200 ml of distillate is used. Any standard 500 ml distilling flask with side arm midway of the neck and assembled as previously described¹ is satisfactory.

It is recommended that the simplified distillation procedure, followed by a chromatographic separation of the isolated volatile acids, be studied collaboratively.

Preliminary work on water-insoluble fatty acids in fish and fish products indicates that this may be another method for the detection of the use of decomposed material. Likewise, succinic acid has been found to offer similar possibilities in fish.

No reports were given for decomposition in shellfish, or in apple products.

A contributed paper, entitled "Water-insoluble Fatty Acids in Cream and Butter," by Fred Hillig and S. W. Ahlmann, was published in *This Journal*, 31, 739 (1948); and a paper entitled "Volatile Acids in Cream and Butter," in two sections, "Part I, The Development of Butyric Acid During the Progressive Decomposition of Cream," by Fred Hillig, and "Part II, Butyric Acid in Commercial Creams and Butters," by Fred Hillig and Dorothy Montgomery, was published in *This Journal*, 31, 750 (1948).

¹ *Methods of Analysis*, A.O.A.C., 6th Ed., p. 361 (1945).

² *This Journal*, 28, 644 (1945).

REPORT ON GELATIN, DESSERT PREPARATIONS, AND MIXES

By S. C. ROWE (Food and Drug Administration, Federal Security Agency,
Washington 25, D. C.), *Referee*

Last year your General Referee recommended that collaborative study be undertaken of the jelly strength methods for plain gelatine and gelatine dessert powders in paragraphs 9.6 and 9.12. The Associate Referee on jelly strength is Paul A. Kind of the Kind-Knox Gelatine Company, Camden, New Jersey, assisted by Dr. D. Tourtellotte, of that firm. During the year, the Association was fortunate in securing the services as Associate Referee on Gelatin and Gelatin Desserts (Constituents) of Dr. Joseph H. Cohen, General Manager, Atlantic Gelatin Division, General Foods Corporation.

No report on jelly strength was received in time for this meeting. The recommendation* of last year is repeated, that the methods for jelly strength (*This Journal*, 31, 74 (1948) and paragraph 9.12) be studied collaboratively with a view to making them official. In addition, it is recommended* that the methods for sucrose, dextrose, and starch (paragraphs 9.13, 9.15, and 9.21) be studied collaboratively with a view to making them official for the 1950 Edition of *Methods of Analysis*.

No report was given on jelly strength.

REPORT ON GUMS IN FOODS

By F. LESLIE HART (Food and Drug Administration, Federal Security
Agency, Los Angeles, California), *Referee*

Last year the Association recommended that further work be done on detection of gums in curd cheeses, cacao products, frozen desserts, and salad dressings. Only one formal report was received by the Referee, that of Associate Referee Coulter on detection of gums in mayonnaise and French dressing. The Associate Referee has shown that the amended method, 33.5, adopted as tentative in 1945, and published in *Methods of Analysis*, Sixth Edition, is applicable to the detection of gums in these products, and he recommends its adoption as official after minor changes in wording. Your Referee concurs in this recommendation.

Associate referees assigned to the subjects of cacao products, and soft curd cheeses have both done work during the year, but they have not progressed sufficiently to render a formal report. No work was done on frozen desserts during the year.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

RECOMMENDATIONS*

It is recommended—

(1) That the method for detection of gums in mayonnaise and French dressing, given in *Methods of Analysis*, Sixth Ed. 33.57, be amended by substituting "50 ml" for "1.5 oz." in line 3, paragraph 2, and that the method, as amended, be adopted as official, first action.

(2) That studies be continued on the detection of gums in soft curd cheeses.

(3) That studies be continued on the detection of gums in cacao products.

(4) That studies be continued on the detection of gums and other stabilizers in frozen desserts.

(5) That an Associate Referee be appointed to study detection of gums in catsup and related tomato products.

REPORT ON GUMS IN MAYONNAISE
AND FRENCH DRESSING

By E. W. COULTER (Food and Drug Administration, Federal Security Agency, Chicago 7), *Associate Referee*

The Associate Referee on Gums in Mayonnaise and French Dressing recommended, in 1945, that "the amended method be adopted as tentative and that studies on the detection of gums in Mayonnaise and French Dressing be discontinued." The amendment referred to was a change in the wording which would aid the analyst in differentiating between the precipitate due to added gums and that due to spices. Since no collaborative work was done following this change in wording, Subcommittee C in 1947 recommended that Gums in Mayonnaise and French Dressing be studied.

Collaborative work completed this year is confined to the detection of added gum in mayonnaise. Four different brands of mayonnaise were examined for gums by method 33.57, page 548, *Methods of Analysis*, 6th edition. All four were negative for added gum, but varying amounts of precipitate due to spices were obtained. The brand giving the largest amount was used in preparing samples for collaborative study.

These samples were prepared by adding to the mayonnaise 0.2 per cent by weight of the dry powdered gum. The powder was incorporated into the mayonnaise by thorough hand stirring with a broad spatula. Each batch consisted of sufficient mayonnaise to yield six 225 gram portions. Three gums were used—tragacanth, guar, and carob bean. Each collaborator received four 225 gram subs (one without added gum) and was instructed to test each for gums by Method 33.57.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 55, (1949).

The following table gives the results obtained by six analysts on four samples:

TABLE 1.—*Collaborative results*

SUB	1	2	3	4
Type of added gum.	Trag.	None	Guar	Carob
Conclusions:				
Analyst: S. H. Perlmutter	Present	Absent	Present	Present
H. D. Silverberg	"	"	"	"
H. W. Conroy	"	"	"	"
F. H. Collins	"	"	"	"
E. C. Deal	"	"	"	"
J. H. Bornman	"	"	"	"

ANALYSTS' COMMENTS

S.H.P.—"Sample 2 was difficult to judge, there was a voluminous precipitate in the alcohol, but the Benedicts test was negative and the Molisch test doubtful."

H.D.S.—"Copper reduction test. Voluminous red precipitate in subs 1, 3, and 4. No precipitate in sub. 2."

H.W.C.—"Sample 2 produced a fairly heavy alcohol insoluble precipitate. The confirmatory test with Benedicts was negative and the tests with naphthol and thymol were also negative."

F.H.C.—"Sub 2 with Molisch test gave a positive indication after standing a while. It is suggested that the metric system be used in the 3rd line of the 2nd paragraph of the method. Since the 1.5 oz. figure used is more or less arbitrary the maximum might be satisfactorily changed to 50 ml."

E.C.D.—"Subs 1, 3 and 4 gave a considerable amount of flocculent precipitate and sub 2 a small amount of alcohol insoluble material. A very faint positive Molisch test was obtained on sub 2, but the copper reduction test was negative."

J.H.B.—"No difficulty was experienced with the method. The only question in my mind was 'how much is a slight precipitate?' The slight precipitate obtained in sub. 2 gave negative results."

The above results show that all six analysts agree as to the presence or absence of added gum. One analyst reported for Sub 2 a doubtful Molisch test and one a positive Molisch after standing, but these results did not lead to erroneous conclusions.

RECOMMENDATIONS*

It is recommended—

(1) That the wording of the method be changed by substituting "50 ml." for the "1.5 oz." in the third line of the second paragraph.

(2) That the method, with the recommended change in wording be adopted as official, first action, and that the subject be closed.

No reports were given on gums in cheese, frozen desserts, or cacao products.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 55 (1949).

REPORT ON MEAT AND MEAT PRODUCTS

By R. M. MEHURIN (Bureau of Animal Industry, Meat Inspection Division, Washington 25, D. C.), *Referee*

Since the last meeting, the Associate Referee on Soybean Flour in Sausage and Similar Products has continued work on the method presented last year for the determination of soybean flour in sausage. However, because of press of other work, samples for analysis were sent to collaborators too late to secure results in time for a report at this meeting. A report will be given on this subject at the next meeting.

Preliminary work on the method of Eggleton, *et al.*,¹ referred to last year as possibly suitable for the direct determination of creatine in meat extracts, has given promising results. The method consists in the addition of diacetyl and alphanaphtol to a suitably diluted extract. The solution is allowed to stand about 15 minutes and the absorption at 525 millimicrons wave length read in a photoelectric colorimeter or spectrophotometer. The concentration is read from a standard curve prepared by treating solutions of known creatine content in the same manner. The method is rapid and appears to give good reproducibility. It is now being compared with the official method. Results to date indicate that further study of the method is warranted, with a view to providing a direct method for the determination of creatine in lieu of or in addition to the present method in which creatine is determined by difference. Credit for this preliminary work should go to John M. McCoy, Meat Inspection Division, Department of Agriculture, Washington, D. C.

It is recommended*—

(1) That collaborative work be continued by the Associate Referee on the determination of soybean flour in sausage and similar products.

(2) That an Associate Referee be appointed to continue work on methods for the determination of creatine and creatinine in meat and meat products.

No reports were given on dried skim milk, soybean flour, or creatin in meat products.

No report was given on naval stores.

No report was given on radio activity, including quantum counter.

¹ *Biochem. Journal*, 37, 526 (1943).

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 55 (1949).

REPORT ON NUTS AND NUT PRODUCTS

By A. M. HENRY (Food and Drug Administration, Federal Security Agency, 416 Federal Annex, Atlanta 3, Ga.), *Referee*

Several mechanical devices for preparing nuts and nut products, some of which may be patented, were investigated during the year. While some of them were of value for special products, none were an improvement on an ordinary food chopper for preparing a variety of nuts and nut products.

Further investigations were made of the methods for moisture determinations reported on last year. This work was done by Phyllis B. Rokita and Beulah V. McMullen. The Toluene Distillation Method was modified by washing the apparatus with an aerosol solution and by adding a small amount of aerosol solution to the distillation and receiving flasks in order to prevent the formations of droplets of water. This modification was not satisfactory as droplets of water still formed. This method does not seem to be adaptable to nuts and nut products in general.

A considerable amount of work was done to ascertain the minimum drying time for various nut products. Most nut meats will come to an equilibrium after two hours drying, although some nut products, such as sweetened grated coconut, require five hours. Additional drying of ten to fifteen hours does not make any appreciable difference unless decomposition takes place. The per cent of loss is different under different conditions. For instance, a sample of raw peanuts dried in the vacuum oven for five hours gave a range of 6.92 to 7.01 per cent for several separate determinations, while when dried for ten hours, the range was 6.97 to 7.00 per cent and fifteen hours 7.01 to 7.04 per cent. When this same sample was dried in a mechanical convection oven at 100°C. the loss on drying was 6.61 per cent at two hours, 6.63 per cent at three hours, 6.57 per cent at four hours, 6.63 per cent at five hours, and 6.58 per cent at six hours.

In products with low moisture content, as most nuts have, the use of a covered dish is not necessary. Typical results with covered aluminum dishes and sintered glass extraction tubes as containers on peanuts and pecans are as follows:

TABLE 1.—*Typical results on peanuts and pecans*

SAMPLE NO.	COVERED ALUMINUM DISHES	SINTERED GLASS TUBES
	PER CENT MOISTURE	PER CENT MOISTURE
1	6.99	6.96
2	7.02	6.98
3	7.04	6.99
4	7.00	6.99
5	6.98	6.99
6	6.96	6.98

The drying of nuts at 100° in air, 100° in nitrogen, and 100° in vacuum was compared. The following results on a sample of peanuts is typical of this investigation:

At 100° in air, 6.70 per cent.
At 100° in nitrogen, 6.70 per cent.
At 100° in vacuum, 6.92 per cent.

Further investigation was made by Mrs. Rokita and Miss McMullen on the methods for fat determination reported on last year. The methods for fat in chocolate liquor, as given in 19.23 and 19.24 were further studied. These methods do not seem to be applicable to most nut materials, as the fat is not completely extracted from such materials without first extracting nearly to completeness and then regrinding the material and final extraction with several portions of ether.

The method for fat as given in 30.6 was modified by making the chloroform extraction up to a definite volume and taking an aliquot. This modification was an improvement over the original method. This modification does not give satisfactory results on products with very high fat content as in many nut meats.

The Soxhlet method 27.24 and 27.25 gave very good results. Determination on one sample of peanuts gave the following results for fat: 44.33, 44.39, 44.31, 44.27, and 44.13, average 44.30. Most peanut meats gave some trouble with this method as fine starch comes through most filtering mediums. This can be prevented by care in selecting and preparing the filtering mediums.

All the methods for Nuts and Nut Products were adopted as "tentative" in 1935 following the Referee's recommendation in 1934. These methods were an adaptation of methods used for other products that had appeared in chemical literature and of special methods developed by individuals for particular adulterations in certain products. No further investigational or collaborative work has been done on them. In 1947 the Referee made a preliminary investigation on the methods for moisture and ether extract, which indicated that such methods should be of a broad general application, as nuts vary greatly in the percentage of moisture and ether extract and the oils in the ether extracts vary greatly in the characteristics of the oils from pecans to cocoanuts.

Not only were the "tentative" and other methods discussed with Federal and State Control Officials and Federal and State Chemists doing research work, but they were also discussed with chemists interested in the examination of nuts and nut products in "industry."

As a result of these conferences and discussions, and the suggestion of the Committee on Classification of Methods, a careful study was made of the whole chapter. The chapter has been rewritten.¹ Methods of Preservation and Preparation of Samples are added. A number of methods

¹ Details of some of the tentative methods proposed are published in *This Journal*, 32, 96 (1949).

for determinations of general type which have been studied and adopted for many other food products are applicable to the analysis of nuts; and several methods have been listed for study, which are believed to be desirable for the chapter, including a sorting method, a method for added starch in nut butters and pastes, and added glycerol and glycols.

RECOMMENDATIONS*

It is recommended—

(1) That methods for preservation of samples and preparation of samples be adopted.

(2) That methods for moisture, crude fat, crude protein, crude fiber ash, reducing sugars, sucrose, sodium chloride, and water-insoluble inorganic residue be adopted as official, first action.

(3) That methods for added coloring matters; metals, other elements, and residues; and preservatives and artificial sweeteners, be adopted by reference to the appropriate chapters.

(4) That sorting methods for moisture and fat, and methods for added starch in nut butters and pastes, and added glycerol and glycols be studied.

REPORT ON VEGETABLE DRUGS AND THEIR DERIVATIVES

By PAUL S. JORGENSEN (Food and Drug Administration, Federal Security Agency, San Francisco 2, California), *Referee*

RECOMMENDATIONS†

(1) *Chemical Methods for Ergot Alkaloids*.—No report was received. It is recommended that the subject be continued.

(2) *Physostigmine in Ointments*.—The Associate Referee recommends that the proposed method be adopted as tentative. The Referee recommends that the method be adopted as official, first action.

(3) *Theobromine and Phenobarbital*.—The Associate Referee submitted a report and recommended that the subject be continued. The Referee concurs in this recommendation for the purpose of developing the spectrophotometric method.

(4) *Aminopyrine, Ephedrine, and Phenobarbital*.—No report was received. It is recommended that the subject be continued.

(5) *Quinine*.—The Associate Referee recommended that the subject be continued. The Referee concurs in this recommendation for the purpose of submitting the Herd procedure to collaborative study to determine if it should be made official.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 56 (1949).

† For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

(6) *Chemical Methods for Penicillin*.—No report was received. The Referee recommends that the subject be continued.

(7) *Rutin in Tablets*.—No report was received. The Referee recommends that the subject be continued.

(8) *Ethylmorphine in Syrups*.—The Associate Referee recommends that the usual alkaloidal assay procedure be used to determine ethylmorphine in syrups in the absence of other alkaloids, and that this procedure be submitted to collaborative study. The Referee concurs in this recommendation.

(9) *Arecoline Hydrobromide*.—The Referee recommends that method 39.73 be made official, first action.

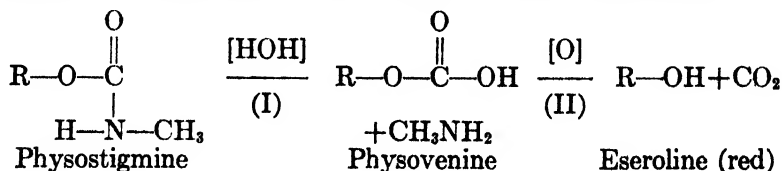
REPORT ON PHYSOSTIGMINE IN OINTMENTS

By MATTHEW L. DOW (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

The work of the previous year indicated that the official A.O.A.C. titration method (1) for physostigmine salicylate might apply also to ointment preparations if some method for completely extracting the alkaloid from the base could be found. Accordingly, several variations of a direct acid extraction procedure were tested, using as controls carefully weighed amounts of the pure alkaloid added to a 1-9 lanolin-petrolatum base. An alternate melting and chilling operation in an Erlenmeyer flask containing a glass stirring rod for mixing and transfer purposes was found to give consistent recoveries of 95-98 per cent when the extracted alkaloid was titrated by 39.99. Despite the most careful technic it was never possible to recover 100 per cent of the alkaloid except by the direct extraction of an aqueous solution of the pure salt.

A very sensitive colorimetric procedure suggested by Shupe (2) based on the formation of a blue compound with nitrous acid and strong potassium hydroxide, was investigated, in the hope that better results would be obtained. This method proved to be valuable in the study of the stability of physostigmine in solution, but it could not be used to determine the original amount of alkaloid in a preparation.

Apparently physostigmine decomposes in solution as follows:



Step (I) was found to take place slowly in warm 2% sulfuric acid. By means of controls, prepared immediately before extraction, it was determined that from 5 to 15 per cent of the alkaloid salt added to the oint-

ment hydrolyzed during the proposed extraction procedure. In dilute alkaline sodium bicarbonate solution, both the hydrolysis and oxidation steps were found to proceed rapidly, unless the solution was kept cold (less than 15°C).

Since the blue color is formed only with secondary alkyl urethanes, only the unchanged physostigmine in the extract can be measured by this colorimetric method. On the other hand, both combined and free methylamine can be titrated by 39.99. Therefore, since methylamine is extracted completely along with the alkaloidal salt, the original amount of physostigmine added can be determined in this way.

Since the titration method determines the unchanged physostigmine plus any methylamine resulting from hydrolysis, either before or during extraction, and since the colorimetric procedure determines only the unchanged alkaloid in the extracted solution, which has already undergone some hydrolysis, it is not possible by the methods now at hand to determine exactly the amount of unchanged physostigmine in an ointment. The Associate Referee now has on hand several ointments made up during the past few years. Analysis of these ointments by both methods should show whether there is appreciable hydrolysis of the alkaloid on aging, and whether the resultant methylamine leaves the ointment.

Two ointments prepared in 1944 and 1947, respectively, and stored at room temperature until examination, were analyzed by the proposed titration method and found to have lost about 10 per cent in the case of the former, and approximately 5 per cent in the case of the latter, of the added amounts of physostigmine salicylate. Apparently some methylamine escapes from the ointment. It is intended to repeat the work by both methods, to determine the extent of the hydrolysis.

Table 1 gives the results of a series of recovery experiments from freshly prepared controls in which a carefully weighed amount of physostigmine salicylate was thoroughly mixed with approximately 5 grams of ointment base and immediately extracted and titrated by the proposed method.

TABLE 1.

DETERMINATION	% RECOVERY
1	97.1
2	96.9
3	97.5
4	96.7
5	95.2

A carefully prepared ointment containing 0.25 per cent of physostigmine salicylate was submitted for collaborative study.¹ Results are shown in the table below.

¹ Details of the proposed method are published in *This Journal*, 32, 113 (1949).

TABLE 2.—*Collaborative results*

COLLABORATOR	PHYSOSTIGMINE SALICYLATE FOUND	RECOVERY
	per cent	per cent
1	0.19	76.0
	0.20	80.0
	0.22	88.0
2	0.24	96.0
	0.25	100.0
	0.24	96.0
3	0.24	96.0
	0.25	100.0
	0.24	96.0
4	0.24	96.0
	0.25	100.0
5	0.25	100.0
	0.25	100.0
	0.25	100.0

COMMENTS OF COLLABORATORS

Rupert Hyatt, Cincinnati, Ohio.

Accurate weighing is not so necessary with a small proportion of physostigmine. I suggest that a beaker be listed as optional because it is hard to put the ointment in a flask without smearing the neck, unless a piece of glassine paper is used. The test for complete extraction could be eliminated by requiring an additional shakeout.

Henry R. Bond, Kansas City, Mo.

No operational difficulties encountered. The titration is so small (1 ml) that there is a difference of 5% in recovery per drop of N/50 acid or alkali. Perhaps more accurate results would be obtained if .005 N solutions were used.

The Associate Referee is indebted to the following members of the Food and Drug Administration for their valuable comments and participation in this work: H. R. Bond, Kansas City, Mo.; Rupert Hyatt, Cincinnati, Ohio; G. S. Keppel, Minneapolis, Minn.; and Mary McEniry, St. Louis, Mo.

DISCUSSION

The results obtained by four of the five collaborators were excellent, both from the standpoint of recoveries obtained and replicability. The recoveries of the fifth collaborator were not only low as compared to the others but also showed considerable variance.

It is recommended* that the proposed method be adopted as tentative.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

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- (2) I. W. SHUPE, *Cosmetics and Color*, 9, 12 (1941).
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REPORT ON THEOBROMINE AND PHENOBARBITAL

By DANIEL BANES (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In studying methods for the analysis of mixtures containing theobromine and phenobarbital Deal showed¹ that the two drugs could be separated by the use of ether and dilute acid as immiscible solvents. Later Richardson and Campbell described² a procedure for the determination of theobromine by precipitation as the phosphotungstate from acid solution, during prolonged digestion on the steam bath. It has been found that the heating period can be materially reduced, with the production of larger crystals, by boiling the acidified theobromine-phosphotungstate mixture.

To test the efficiency of the shake-out separation, and the accuracy of the modified method for the alkaloid, a prepared mixture consisting of theobromine (20.44%), phenobarbital (7.34%), and starch was subjected to collaborative study. Instructions for procedure and results of analysis are shown below.

METHOD

Transfer a portion of the well-mixed sample containing at least 100 mg phenobarbital to 125 ml separatory funnel, add 10 ml of 5% NaOH and extract with three 30-ml portions of CHCl_3 . Wash the CHCl_3 layers successively with 5 ml of 5% NaOH in a second separator. Reject the chloroformic extracts.

Add 30 ml of H_2SO_4 (1+4) to the alkaline mixture in the first separator, cool, and shake vigorously with 50 ml of ether. Transfer the aqueous layer containing dissolved theobromine to the second separator, cool, and shake with 35 ml of ether. Remove the lower phase to a third separator and wash with another 35 ml of ether. Repeat the extraction thru the three separators using two 40 ml portions of H_2SO_4 (1+4), and three 20 ml portions of water. Collect the aqueous layers in 250 ml volumetric flask, dilute to the mark with water and mix.

Theobromine.—Transfer an aliquot containing 100–200 mg of theobromine to a 250 ml beaker, dilute to 100 ml with water, and stir in 10 ml H_2SO_4 (1+1), and 10 ml of a clear 20% soln of phosphotungstic acid crystals in water. Cover the beaker with watch glass and partially immerse in glycerine bath. (A 600-ml beaker half-filled with glycerine is a convenient vessel.) Heat to gentle simmering by maintaining a bath temperature of 115–130°, and stir frequently until the precipitate subsides as a lemon-yellow crystalline mass and the supernatant liquid becomes clear (usually 20 min.). Digest at boiling point an additional hour with occasional stirring. Filter hot with suction thru a tared Gooch crucible. Transfer precipitate to the crucible quantitatively with the aid of rubber policeman and ten 10 ml portions of cold HCl

¹ Deal, E. C., *This Journal*, 24, 818–20 (1941).

² Richardson, A. G., and Campbell, Y. C., *J. Am. Pharm. A.*, 31, 24–26 (1942).

(1+9). Aspirate several minutes, dry to constant weight at 120°, cool and weigh. The weight so obtained $\times 0.1563$ is the weight of theobromine in the aliquot. Potassium iodide interferes.

Phenobarbital.—Filter the ethereal soln thru a pledget of cotton into a tared beaker, washing the three separators and the filter successively with three 5 ml portions of ether. Evaporate to dryness on steam bath with aid of a current of air, heat to constant weight at 110°C. and weigh as phenobarbital. In the presence of stearic acid, proceed as directed in *Methods of Analysis*, 1945, Sec. 39.49.

TABLE 1.—Recoveries of theobromine and phenobarbital

COLLABORATOR	THEOBROMINE		PHENOBARBITAL	
	FOUND	RECOVERY	FOUND	RECOVERY
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
A. G. Buell	19.91	97.8	7.43	101.2
	19.81	96.9	7.45	101.5
A. W. Steers	19.86	97.2	7.44	101.4
	19.83	97.0	7.45	101.5
C. R. Joiner	19.45	95.2	7.10	96.7
	19.43	95.1	7.09	96.6
D. Banes	20.15	98.6	7.31	99.6
	20.20	98.8	7.36	100.3
R. D. Stanley	19.77	96.7	7.19	98.1
	19.69	96.3	7.22	98.5
H. Isacoff	20.5	100.2	7.4	101.0
	20.5	100.2	7.3	99.6
Average	19.93	97.5	7.31	99.7

DISCUSSION AND RECOMMENDATIONS

While recoveries of theobromine are somewhat low, due, perhaps, to the solubility of its phosphotungstate in dilute acid, the method appears to be promising. Results for phenobarbital are particularly encouraging.

Richardson and Campbell state² that potassium iodide interferes in the gravimetric method for theobromine. Since both that alkaloid and phenobarbital absorb ultra-violet light, a spectrophotometric procedure was devised in an attempt to obviate the difficulty. Collaborative results indicated weaknesses in the method, and it has not been included in this report.

It is recommended* that the problem be subjected to further study.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949.)

REPORT ON QUININE

By DAVID J. MILLER (Food and Drug Administration, Federal Security Agency, Buffalo, New York), *Associate Referee*

The acidimetric titration of quinine by the A.O.A.C. method (1) has been criticized because of difficulty in determining the end point. Herd (2) ascribed this difficulty to the buffering action of the monoacidic salt first formed, and to overcome this difficulty titrated quinine in glacial acetic acid solution with standard perchloric acid in glacial acetic acid, following the theory developed by Conant, Hall, and Werner, and Nadeau and Braucher. In addition to the perchloric acid titration Herd outlined a procedure for the separation of quinine and strychnine using dichloroacetic acid in chloroform. It is the purpose of this preliminary report to compare the Herd perchloric acid titration with the method now official in the *Methods of Analysis* using pure quinine alkaloid only. At the same time there is presented a third procedure, developed by the Associate Referee, in which quinine is titrated with standard sulfuric acid using the blue fluorescence which appears after the formation of the normal salt and which is easily apparent under ultra violet light, to indicate the end point.

EXPERIMENTAL

REAGENTS

The quinine used in the study was a Mallinckrodt U.S.P. XI Product. Since the purpose of the study was to compare simplicity of titration and recoveries, rather than to determine exact quinine content, no attempt was made to purify the alkaloid. The quinine was passed through a 30-mesh screen and dried at 105°C before use. The melting point (uncorrected) was 175–176°C.

0.1 *N* and 0.02 *N* sulfuric acid, 43.14

0.1 *N* and 0.02 *N* sodium hydroxide, 43.2

0.1 *N* and 0.02 *N* perchloric acid in glacial acetic acid was prepared as outlined by Herd (*loc. cit.*) and standardized against anhydrous sodium acetate, recrystallized α -naphthylamine, and an especially purified quinine obtained from Chemical Section, Medical Division, U. S. Food and Drug Administration, Washington, D. C.

α -naphtholbenzein indicator, 0.2 g/100 ml glacial acetic acid

Bromocresol purple indicator, 39.11

Glacial acetic acid, ACS.

METHODS

Method I is the present A.O.A.C. method. The sample is dissolved in 5 ml neutral alcohol and titrated to a yellow end point using bromocresol purple indicator, the alcohol evaporated and the solution further titrated if there is any change from the yellow. Approximately 0.3 ml of 0.02 *N* acid is consumed from the first color change to a final yellow.

Where 0.02 *N* acid is used to titrate approximately 0.1 g quinine, it is

possible to follow the directions in *Methods of Analysis* but the end point is somewhat difficult to detect. However, the end point can be improved by adding a slight excess of acid, evaporating the alcohol, cooling, filtering, washing, and back titrating with 0.02 *N* alkali, using the first change from yellow as the end point. Where 0.1 *N* sulfuric acid is used to titrate

TABLE 1.—Per cent quinine in sample by titration with 0.02 *N* solutions

METHOD I		METHOD II	METHOD III	
DIRECT* TITRATION	BACK† TITRATION	DIRECT TITRATION	DIRECT TITRATION	BACK TITRATION
99.9	100.4	100.2	97.5	99.3
100.3	99.8	99.6	97.6	97.2
98.8	100.2	99.9		98.3
99.5	99.7	99.7		99.2
100.3	100.9	99.6		99.2
98.9	100.7	100.1		98.0
Av. 99.6	100.3	99.9	97.6	98.5

* A.O.A.C. method exactly.

† Excess acid added to same sample and back titrated as described under "Methods."

TABLE 2.—Per cent quinine in sample by titration with 0.1 *N* solutions

METHOD I		METHOD II	METHOD III	
DIRECT* TITRATION	BACK† TITRATION	DIRECT TITRATION	DIRECT TITRATION	BACK TITRATION
100.5		100.5	99.6	99.1
98.8		100.4	99.4	99.3
99.8	100.1	100.0	98.1	99.3
101.7	100.3	100.1	100.1	99.1
	99.6	100.4		98.2
	99.2	100.5		
Av. 100.5	99.8	100.3	99.3	99.0

* A.O.A.C. method plus addition of 15 ml H₂O.

† Excess acid added and back titrated as described under "Methods."

approximately 0.5 g of quinine the voluminous precipitate of the sulfate makes an accurate titration almost impossible if the A.O.A.C. directions are followed exactly; however, a direct titration can be made if approximately 15 ml water is added and the solution is heated. Approximately 0.25 ml 0.1 *N* acid is used from the first color change to a final yellow. Here, too, it is preferable to add an excess of acid, evaporate the alcohol, etc., and back titrate. Tables 1 and 2 include the results obtained by using the official method exactly and the back titration procedure.

Method II is the Herd perchloric acid titration method. The sample is

dissolved in 20 ml glacial acetic acid and titrated with standard perchloric acid in glacial acetic acid to a green end point using α -naphtholbenzein indicator. It is necessary to determine the temperature of the standard solution and make a correction (approximately one part in a thousand for every degree C difference) if the temperature is different from the one at which it was standardized. Approximately 0.25 ml of 0.02 *N* acid titrating 0.1 g quinine or 0.20 ml of 0.1 *N* acid titrating 0.5 g quinine is used from the first color change to a final green.

Method III consists of the titration of quinine dissolved in 5 ml neutral alcohol with standard sulfuric acid, using as the end point the appearance (or, if back titrating, the disappearance) of a blue fluorescence under ultra violet light. This point corresponds to the formation of the normal sulfate $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$. It is necessary to make the titration in the dark since the fluorescence is not sufficiently marked, even under ultra violet, if much daylight is present. At the end point there is an interval of approximately 0.15 ml of acid, either 0.1 *N* or 0.02 *N*, where there is some question as to the presence of fluorescence. Although the change both by direct titration and back titration is marked it appeared somewhat easier to back titrate, noting the change from fluorescence to absence of fluorescence. Halides and acetophenetidin interfere with this titration.

Results are shown in Tables 1 and 2.

CONCLUSIONS

On the basis of this preliminary study it is concluded that the recoveries of quinine are about equal by the Herd perchloric acid titration and the A.O.A.C. method. The Herd procedure is somewhat superior to the A.O.A.C. method in that with a direct titration the end point is sharper. However, this superiority is not so marked that it outweighs the disadvantage of having to keep prepared still another standard solution which is somewhat unpleasant to use and whose temperature correction is much larger than for aqueous solutions. The titration of quinine, using its fluorescence in sulfuric acid solution as an indicator, is a simple and interesting method of titration, but with pure quinine it offers no particular advantage over either of the other two methods. With impure, colored residues its usefulness may be enlarged.

It is proposed to study the Herd procedure for the separation of quinine and strychnine and it is recommended* that the subject be continued.

REFERENCES

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* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

REPORT ON ETHYLMORPHINE IN SIRUPS

By F. J. McNALL (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

In accordance with the recommendation of Subcommittee B of the Association (*This Journal*, 31, 46) a preliminary study was made for the determination of ethylmorphine in sirups.

Ethylmorphine hydrochloride ($C_{15}H_{23}O_2N \cdot HCl \cdot 2H_2O$), a synthetic alkaloid salt commonly known as Dionin, is used internally in a sirup vehicle, for the relief of excessive cough and pain in the chest. A review of cough sirups on the market containing ethylmorphine showed that, in addition to ethylmorphine, many contained other alkaloid-bearing drugs, such as ephedra, lobelia, ipecac, cocillana, and sanguinaria.

A review of the literature indicated that very little work had been done on the quantitative determination of ethylmorphine. Only one reference was found relative to the separation of ethylmorphine in the presence of other alkaloids. This article "Chromatographic Analysis of Alkaloidal Salts," is reported by F. Reimers and K. R. Gottlieb, in *Chem. Zentr.* II, 1387 (1943).

Ethylmorphine in a simple sirup in the absence of other alkaloids may easily be determined by the usual alkaline chloroform shake out. 30 mg. added to a simple sirup was extracted with a recovery of 98.7 per cent. Sodium hydroxide, which is used to separate morphine from other alkaloids, was tried with a mixture of codeine and ethylmorphine without success.

Alkaloids found in cough sirups other than ethylmorphine are usually present in very small amounts, and as such would not appreciably affect the quantitative estimation of ethylmorphine.

It is recommended* that the usual alkaloidal assay method be submitted for collaborative study for the determination of ethylmorphine in sirups in the absence of other alkaloids.

No reports were given on chemical methods for ergot alkaloids, aminopyrine, ephedrine and phenobarbital, chemical methods for penicillin, or rutin in tablets.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

REPORT ON SYNTHETIC DRUGS

By F. C. SINTON (Food and Drug Administration, Federal Security Agency, New York, N. Y.) *Referee*

RECOMMENDATIONS*

(1) *Butacaine Sulfate*.—A preliminary report was received by the Referee. Some exploratory work was performed and it was recommended that the subject be continued. The Referee concurs.

(2) *Propadrine Hydrochloride*.—No report. The Referee recommends that the subject be continued.

(3) *Pyribenzamine and Benadryl*.—No report. The Referee recommends that the subject be continued.

(4) *Carbromal*.—No formal report was received but the Associate Referee in correspondence indicated that he has worked on two methods involving bromine determination. These appear satisfactory and he is ready to submit samples for collaborative study. The Referee recommends that the study be continued.

(5) *Methylene Blue*.—A report was received describing procedures for the determination of methylene blue in compound tablets. The Associate Referee recommended that a collaborative study be made. The Referee concurs.

(6) *Synthetic Estrogens*.—No report. The Referee recommends that the subject be continued.

(7) *Propyl-Thiouracil*.—No report. The Associate Referee has resigned from the Food and Drug Administration. Some preliminary work was performed in an attempt to apply the method for thiouracil recommended for adoption last year. This was not successful. The Referee recommends that the subject be continued.

(8) *Spectrophotometric methods*.—No report. The Referee recommends that the subject be continued.

(9) *Phenolphthalein in Chocolate Preparations*.—No report was submitted by the Associate Referee; it is recommended that the subject be continued.

(10) *Sulfanilamide Derivatives*.—No report. The Referee recommends that the subject be continued.

(11) *Trichloroethylene*.—The Associate Referee has submitted a report and recommends that the method which was studied collaboratively be adopted as official, first action, and the subject closed. The Referee concurs.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

REPORT ON METHYLENE BLUE IN COMPOUND TABLETS

By HARRY O. MORAW (Food and Drug Administration, Federal Security Agency, Chicago 7, Ill.), *Associate Referee*

The 1948 work on the subject was a continuation of unreported investigational work of 1944, 1945, and 1946, on the problems of separation and determination in coated tablets of complex mixtures of essential oils, resins, balsams, powdered plant materials, plant extracts, excipients, and lime carbonate, sugar, and color in the coating.

The present A.O.A.C. dichlorhydrin extraction method was developed for application of a suitable volumetric method of determination. It does not provide for complete separation of water-insoluble material and may permit small amounts of dichlorhydrin, carbon tetrachloride, and water-soluble material to be carried over. The line of separation between layers of dichlorhydrin and water in the beginning of extractions is indistinguishable. The supply of dichlorhydrin available at the time of this recent work tended to dissolve or remain suspended in the water layer to an objectionable extent. Therefore, extractions by this method unless further treated are not suitable for application of a gravimetric method, or a volumetric method in which the contaminants might enter into the reaction.

In view of the above, it was necessary to experiment with reagents and solvents for dissolving or separating which would not interfere with the determination. Hence, concurrent trials of both method of dissolving and methods of determination were made on unmixed known methylene blue and authentic tablet mixtures.

A major difficulty in dissolving the methylene blue in such mixtures as the above is that of knowing when it is completely dissolved. The intense blue color of its solutions prevents observation of solid material. Moreover, the methylene blue may be embedded in or absorbed by some, or a combination, of the tablet ingredients during the manufacturing or pulverizing for analysis.

Because of this difficulty in observing lines of separation between water and solvent layers, the possibility of reducing methylene blue to the leuco base and extracting the latter was considered. It was found that the methylene blue in a commercial sample of tablets containing the ingredients mentioned above could be reduced in 10 to 15 minutes with alkaline hydroxylamine and apparently completely extracted with 4 or 5 50-ml portions of ether. Completeness of extraction can be shown by absence of blue on acidifying a few ml of the reducing mixtures. It would appear that if the reduced compound could be restored to methylene blue by an acid shake-out from the ether, leaving oils and similar materials in the ether, a most desirable quick and direct method of separation would be available. Some further work on this appears to be justified. Since the methods thus far tried for separating or dissolving methylene blue in such mixtures were not satisfactory, attempts were next made to develop a

direct solution method utilizing the extreme solubility of the methylene blue in methanol. It was found that most of the color could be separated from the solid material by triturating repeatedly with methanol. The undissolved solid material was then further extracted and disintegrated by grinding in a mortar after treatment with dilute hydrochloric acid and methanol until practically colorless. From this investigation it appeared to be practical to extract a sample of the ground tablet mixture containing 0.2 to 0.25 g of methylene blue using 20 ml of methanol and 40 ml of 5% hydrochloric acid and making to a volume of 200 ml or equivalent to 10% methanol and 1% hydrochloric acid (gas). After filtering, this solution could be used for determinations by the gravimetric perchlorate and spectrophotometric methods. It was found by trials on known straight methylene blue that these amounts of the reagents did not affect the accuracy of the determinations by these methods. Results are given in Table 1.

The following two solution methods were next tried on authentic tablet mixtures:

(1) Methanol—HCl-water treatment on ground tablets (without previous extraction).

(2) Methanol—HCl-water treatment after dry ether extraction of the oils, resins, etc.

Using solution method (1) there was a tendency for lumps to form in some instances. This appeared to be due to too rapid addition of the hydrochloric acid and methanol. Also the methylene blue crystallized out after making up to volume. Notwithstanding some instances of complete recovery, the low results in other determinations are believed due to incomplete solution. These difficulties were not encountered with solution method (2). This is based on more logical practices, but requires more care in manipulation to avoid losses.

The authentic tablet mixtures were prepared by adding approximately 2 grams of tablet mass to an accurately weighed amount of methylene blue (0.2 to 0.25 g) of known purity and moisture content, and mixing thoroughly. The table mass consisted of the following ingredients in the approximate proportions used in this type of tablet:

Extract kava, powdered cubeb, powdered nutmeg, copaiba, oil of santal, oil of cinnamon, starch, lactose, sucrose, calcium carbonate, talc.

Results on these experiments are given in Table 2.

Methods of determination studied were as follows:

(1) *Volumetric Silver Nitrate*¹—based on det. of Cl displaced when methylene blue perchlorate is formed.

(2) *Volumetric Dichromate*²—based on precipitation by excess dichromate and titration of the excess by thiosulphate.

(3) *Modified U.S.P.* gravimetric perchlorate.

(4) *Spectrophotometric*.

¹ Maurina and Deahl, *J. Am. Pharm. Assoc., Sci. Ed.*, XXXII, 11, 301 (1943).

² Ferrey, G. J. W., *Quart. J. Pharmacol.*, 16, 208 (1943).

TABLE 1.—*Determinations on known methylene blue (unmixed)*
Modified perchlorate and spectrophotometric methods

DET. NO.	METHYLENE BLUE ANHYDROUS 100% BASIS						
	USED	FOUND				VARIATIONS TRIED:	
		BY PERCHLORATE		BY SPECTROPHOTOMETRIC		CRUCIBLES PREWASHED	REAGENTS PRESENT DURING PRECIPITATION; TIME FOR PPTN.
	g	g	per cent	g	per cent		
113-1	.0912	.0921	101.0			No	pptn. in 10% methanol 1% HCl. 10 min. for pptn.
113-2	.0932	.0937	100.5			No	do. 10 min. for pptn.
113-3	.0849	.0866	102.0			No	No methanol or HCl used. 10 min. for pptn.
114-1	.1145	.1159	101.2			No	pptn. in 10% methanol 1% HCl stood 30 min. for pptn.
114-2	.1279	.1291	100.9			No	do. stood 45 min. for pptn.
114-3	.1149	.1162	101.6			No	do. stood 50 min. for pptn.
114-4	.1810	.1814	100.2	.188	103.8	No	do. heated 1 hr. on steam bath with HCl CH ₃ OH before pptn.
115-1	.0980	.0981	100.1			Yes	do. 10 min. for pptn.
115-2	.0856	.0848	99.1			Yes	do. 75 min. for pptn.
115-3	.1024	.1030	100.6			Yes	do. 30 min. for pptn.
115-4	.0865	.0870	100.6			Yes	No methanol or HCl used. Stood 10 min. for pptn.
152-1	.2000	.1996	99.6	.199	99.5	No	10% methanol 1% HCl
118-1	.2284	.2274	99.6	.2254	98.7	Yes	do.
118-2	.2079	.2076	99.8	.207	99.6	Yes	do.
118-3	.1016	.1018	100.2			Yes	do.
118-3	.1016	.1004	98.8			Yes	10% methanol 1.5% HCl
118-3	.1016	.1011	99.5			Yes	10% methanol 1% HCl
118-4	.2038	.2054	100.8	.205	100.6	Yes	do.

TABLE 2.—*Determinations on authentic tablet mixtures*

DET. NO.	METHYLENE BLUE ANHYDROUS 100% BASIS						METHOD OF SOLUTION
	USED	FOUND BY METHODS					
		BY MODIFIED PERCHLORATE % RECOVERY		BY SPECTROPHOTOMETRIC % RECOVERY			
153-1	.2000	.1862	93.1	.186	93.0	Method (1)—Methanol-HCl-oils not removed by ether-reagents added rapidly.	
153-2	.1694	.1696	100.1	.170	100.4	Method (1)—do. Reagents added fractionally.	
154-3	.2000	.1862	93.1	.188	94.0	Method (1)—CH ₃ OH-HCl mixed and added piecemeal.	
154-4	.1710	.1672	97.7	.168	98.3	Method (1) reagents added slowly. soln. stood 2 days. M.B. crystallized out; redissolved by warming.	
155-5	.1700	.1692	99.5	.168	98.8	Method (1)—Methanol and 5% HCl added alternately in small portions.	
155-6	.1728	.1680	97.2	.173	100.0	Method (1)—Heated 30 min. at 80-90°C. with methanol and HCl.	
156-7	.1945	.1908	98.1	.193	99.2	Method (1) methanol and HCl added piecemeal; lumps formed during solution. Stood overnight; M.B. crystallized out; redissolved.	
157-1	.2000	.1944	97.4	.187	93.5	Method (2)—ether soluble removed before dissolving in methanol-HCl.	
166-8	.1840	.1804	98.0	.184	100.0	Method (2)—ether solubles removed by dry extraction. Color exhausted with methanol followed by HCl-methanol.	
166-9	.2019	.2004	99.3	.198	98.1	do. do.	
167-10	.2008	.1980	98.6	.198	98.6	do. do.	
167-11	.1999	.1960	98.1	.197	98.5	do. do.	
167-12	.1974	.1938	98.2	.195	98.8	do. do.	

Method (1) yielded good results on purified samples of straight methylene blue but somewhat high results on unpurified samples. With solutions obtained from mixtures the end point with Ferric indicator was unsatisfactory. The amount of sample required for determination (0.5 g) would be impractical to handle in mixtures. Method (2) yielded such favorable results on known straight methylene blue samples of 0.1 to 0.5 g that considerable work was done to check its accuracy. When applied to smaller samples, *i.e.*, 45 to 60 mg. low results were obtained. Use of more acid in the flask for the reaction with iodide at first seemed to solve the difficulty. Occasional discordant results suggested substitution of 0.4 to 1% hydrochloric acid for acetic acid for the precipitation with dichromate. This improved the character of the precipitate, but occasional discordant results occurred which indicated that the method was not sufficiently perfected. Moreover, the possible oxidizing action of dichromate on contaminants in extracts from mixtures weighed against further work.

Method (3), the U.S.P. gravimetric perchlorate method, yielded high results for the author on straight methylene blue of known purity. This method provides for the use of 100 ml of methylene blue perchlorate test solution for washing the precipitate. A number of batches of this T.S. have formed additional perceptible but scarcely noticeable precipitates on standing after the original filtration. If not refiltered before use a variable error might be introduced. Some variation in the composition of this T.S. may occur because of the difficulty of recognizing when "a slight permanent turbidity results" when preparing it according to the U.S.P. thus allowing larger excess of methylene blue solution to be added to the perchlorate solution. Recent experience indicates the advisability of specifying the approximate volume of the former to add. Another factor is the absorption by the asbestos of the crucibles of a weighable amount of a blue compound from the 100 ml of the T.S. It was found to average 0.0007 g from freshly filtered T.S. Prewashing of the crucibles and using freshly filtered T.S. are therefore recommended when applying the method to straight methylene blue or solutions, extracts, etc., in which other inherent potential errors are negligible.

Since the U.S.P. method provides for precipitation with potassium perchlorate in water solution, and the solution method reported herein prepares a solution of methylene blue in (1+10) 10% hydrochloric acid-water and 10% methanol, it was necessary to try the perchlorate method with these modifications on methylene blue of known purity. Results of these determinations are reported in Table 1 as well as checks by the spectrophotometric method.

SPECTROPHOTOMETRIC DETERMINATIONS

The solution method developed for methylene blue in compound tablet mixtures requires the use of (1+10) 10% hydrochloric acid-water and

(1+10) methanol-water in preparing the original solution. Hence amounts in the diluted aliquots for the colorimetric determinations vary from 0.003 to 0.01 *N* in hydrochloric acid and 2 to 35 mg methanol per 100 ml. No investigation was made to determine whether ageing of solutions of methylene blue in these reagents affects the colorimetric determination. However, peak absorption values relative to blanks of the respective solvents were determined on solutions of 0.3 mg of 100% anhydrous methylene blue in water in 0.1 *N* hydrochloric acid, in 0.05 *N* hydrochloric acid and in 5 ml of 5% hydrochloric acid (approx. 0.15 *N*) plus 5 ml methanol per 100 ml.

This was found to be approximately 667 $m\mu$ in each case. A plot of concentrations corresponding to *E* values was made for amounts varying from 0.01 to 0.4 mg per 100 ml in the same solutions using a slit width of 0.06. The absorption curve and the graph of the concentration corresponding to *E* value at 667 $m\mu$ for the compound in approximately 0.1 *N* hydrochloric acid appear in Figs. 1 and 2. Concentrations above 0.35 mg and below 0.08 mg per 100 ml generally did not fall on a straight line, indicating a slight deviation from the Beers-Lambert Law. However, the graph was used for the colorimetric determinations in Tables 1 and 2 and appears to be practical.

Some further work should be done on this to determine whether a more favorable reagent strength can be found.

PURIFIED SAMPLE TESTS

The sample used for control tests in this work was prepared by dissolving U.S.P. methylene blue in water, filtering and recrystallizing by evaporating and long standing. Crystals were washed with small portions of water and alcohol and allowed to stand in air several days. They were ground to pass a No. 50 sieve and stored in tight bottles. A more rapid method of crystallization tried on another sample was suggested by Martin, Neuhaus, and Reuter³ utilizing alcohol containing 5% methanol.

Nitrogen was determined by the A.O.A.C. method using both copper sulfate and mercuric oxide catalysts. The results by three determinations as anhydrous methylene blue were 84.28%, 84.47%, 84.60%, average 84.45%. Moisture was determined by heating in vacuo at 100°C. for 3 to 4 hour periods to constant weight. Heating for shorter periods, *i.e.*, 1 hour, caused no change but subsequent heating of the same samples for longer periods caused appreciable changes. Placing of fresh samples in the oven with previously dried samples apparently caused the latter to gain weight. Moisture found in the purified sample averaged 15.37% by three determinations. Keeping air oven temperatures constant at 110°C. as required by the U.S.P. was found impractical. Temperature

³ *Analyst*, 71, 29 (1946).

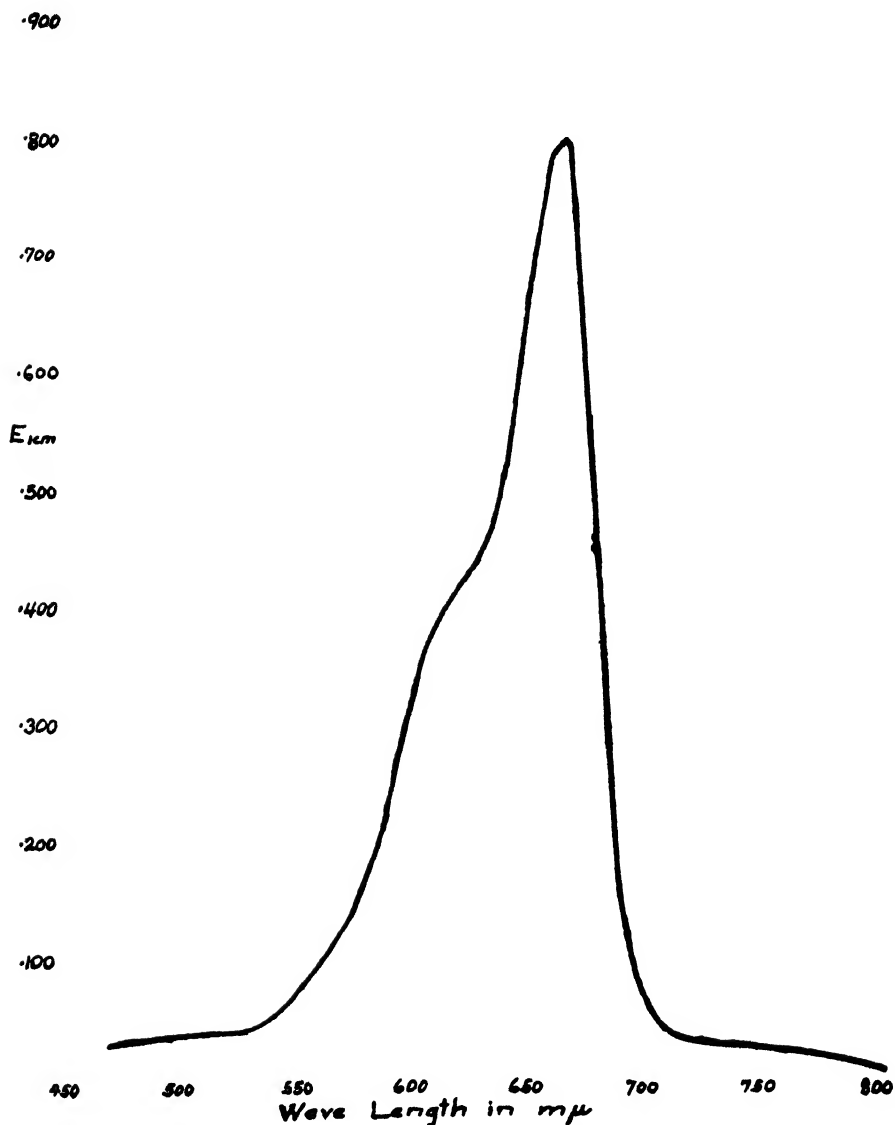


FIG. 1.—The Absorption Spectrum of Methylene Blue in 0.1 N HCl.

variations in the vacuum oven method do not influence the results to the extent they do in the air oven.

The hydrogen peroxide digestion method for nitrogen recommended by Maurina and Deahl¹ in place of sulfuric acid appears to have advantages in saving of time but was not tried. For samples to be used for collaborative study later it is expected to make use of this method if it is found satisfactory.

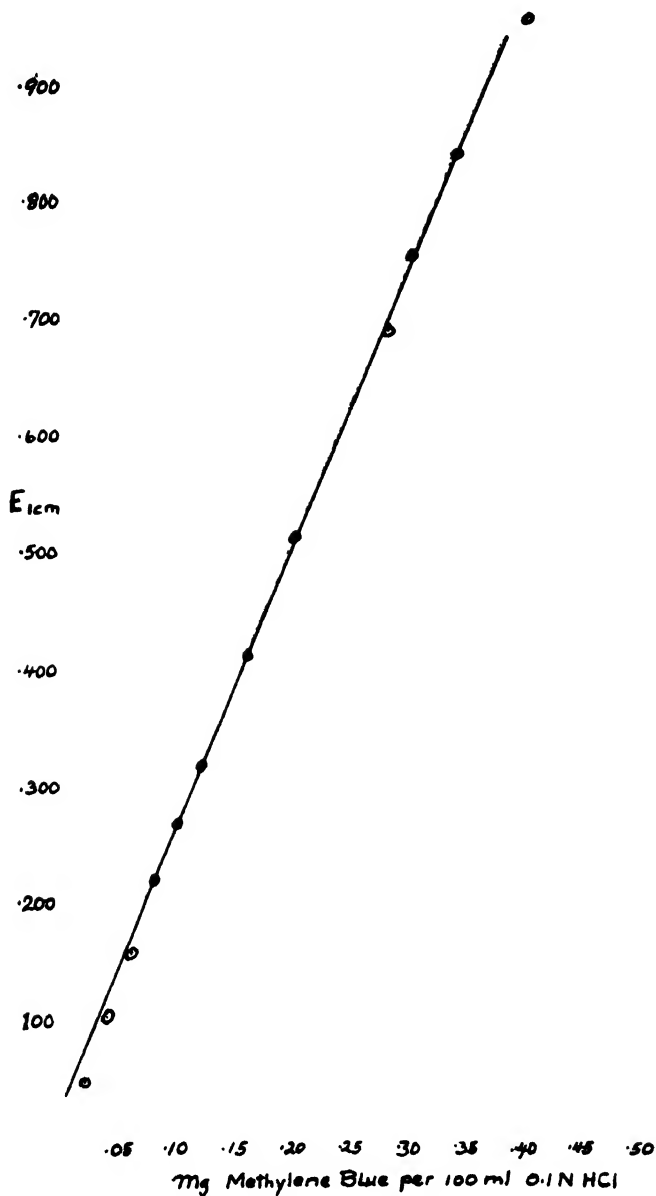


FIG. 2.—Plot of E against Concentration at 667 $M\mu$.

RECOMMENDATIONS*

It is recommended—

- (1) That further work be done to determine if there is a more favorable

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

concentration of hydrochloric acid and methanol for determining the E values corresponding to different concentrations of methylene blue.

(2) That details of solution Method 2 be submitted to collaborators for trials on authentic tablet mixtures and on a sample of commercial tablets of the approximate composition referred to in paragraph 1 of this report. It is also recommended that details of the modified perchlorate and the spectrophotometric methods referred to herein be prepared and tried by collaborators on the authentic tablet mixtures and authentic methylene blue of known purity.

ACKNOWLEDGMENTS

I wish to acknowledge help and suggestions on the colorimetric work received from my associates, Daniel Baner and R. D. Stanley.

No report was made on phenolphthalein in chocolate preparations.

REPORT ON BUTACAINE SULFATE

By LLEWELLYN H. WELSH (Chemical Section, Medical Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.),
Associate Referee

Butacaine, or butyn, is 1-(*p*-aminobenzoxy)-3-dibutylaminopropane, and is used in the form of its neutral sulfate. It appears commercially as the pure salt and in the following dosage forms (N. N. R.): aqueous solution; tablets with and without epinephrine; ophthalmic ointment, one per cent (plain) and two per cent (with metaphen).

From exploratory work in this laboratory, butacaine base appears to be amorphous at room temperature. It is not significantly volatile at 105°C., and may be extracted easily with chloroform from alkaline aqueous systems and weighed. Acid solutions of the substance may be assayed by titrating with bromide-bromate solution which introduces two atoms of bromine into the molecule. The neutral equivalent may be determined by titration with acid to a methyl red end point.

At the present stage of the work, it appears that preparations which will yield sufficient drug, without causing complications due to the nature of the vehicle, may be analyzed by extracting out the base, determining it gravimetrically, and checking the identity of the residue by acid titration followed by bromination of an aliquot of the titrated solution. Products not suited to such a procedure might be analyzed by separating the butacaine by extraction procedures, brominating in acid solution, and identifying the bromination product after isolating it and converting to the hydrochloride or hydrobromide.

This report is of a preliminary nature. In the coming year an effort

will be made to develop a procedure applicable to all dosage forms and to conduct a collaborative study.

It is recommended* that the subject be continued.

REPORT ON TRICHLOROETHYLENE

By GORDON SMITH (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

One of the methods devised by Rauscher¹ for determining organic halogen compounds has been applied this year to trichloroethylene. The central feature of this method consists of heating the sample in a closed tube with monoethanolamine. This converts the chlorine to a form in which it can be precipitated from aqueous solution by silver nitrate. The heating medium used by Rauscher was a bath of boiling diethanolamine, which held the temperature constant at about 268°C. In this work on trichloroethylene a mineral oil bath has been substituted, with the object of greater safety and simplicity. To compensate for the lack of any close automatic temperature control, a rather wide range of temperature is permitted, above the necessary minimum.

It was found that heating at 210°C. or above for 1 hour would give recoveries close to 100%. No attempt was made to determine closely the minimum time and temperature required. One half hour at 200°–220°C. appeared to be insufficient, giving recoveries in the vicinity of 96 or 97 per cent. Heating one hour in the steam bath gave a recovery of only 34 per cent.

The sample used in working out the method was prepared by distilling a commercial product three times, discarding end fractions. The resulting liquid had a density of 1.4562 20°/4° and a refractive index of 1.4774 at 20°. These constants as given in the *Handbook of Chemistry and Physics* for trichloroethylene are, respectively, 1.4556 and 1.4777. Thus the material was regarded as practically 100 per cent trichloroethylene.

Volumetric determination of chloride in the reaction product was first tried, by the usual silver nitrate-thiocyanate method. However, the reaction product has some color, which seems to increase near the end point, making the latter difficult. Results ranged from 98.5 to 99.6 per cent. Gravimetrically, weighing the silver chloride, the recoveries obtained were 99.8, 100.3, 100.1, and 99.6 per cent. One collaborator, Arthur Kramer of the New York Station, also obtained recoveries close to 100 per cent by this method. It was decided to submit the gravimetric method only to collaborative study.

For this purpose, a sample of U.S.P. trichloroethylene was made up,

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

¹ *Ind. Eng. Chem., Anal. Ed.*, 9, 296 (1937).

since it is such a substance that would be assayed in practice; 198.6 g of the pure distillate were mixed with 1.4 g of absolute ethanol; thus the theoretical percentage of trichloroethylene in the sample was 99.3.

All the collaborators were members of the Food and Drug Administration.

Details of the method are given in *This Journal*, 32, 113 (1949).

TABLE 1.—*Collaborative results*

COLLABORATOR	PER CENT TRICHLORO- ETHYLENE	COLLABORATOR	PER CENT TRICHLORO- ETHYLENE
C. F. Buening, Baltimore	99.6 99.3 99.4	L. W. Ferris, Buffalo	94.5 99.6 99.5 99.6
A. Kramer, New York	99.4 99.6	H. P. Eiduson, Buffalo	99.0 99.5 99.3
A. G. Buell, San Francisco	98.4 98.0	L. H. Welsh, Washington	99.9 99.4
A. W. Steers, San Francisco	99.4 99.1 99.4	G. Smith, New York	98.9 99.2 99.1

Most of the results are in close agreement. Of a total of 22 determinations, 17 are in the range 99.0% to 99.6%. The average of these is 99.4%. The average of 21 determinations, omitting the single widely divergent one, is 99.3%.

There was little comment by collaborators. Four saw the need of a filtration step after the reaction product is washed out of the tube, to remove any broken glass. This step has been inserted in the method with the proviso "if necessary." One station reported that several tubes cracked without explosion while being heated.

The method seems accurate enough for practical use. With some study of details it probably could be made more precise and proof against variation.

It is recommended* that the method be adopted as official, first action, and that the subject be closed.

No reports were given on sulfanilamide derivatives, propadrine hydrochloride, carbromal, spectrophotometric methods, thiouracil, pyribenzamine and benadryl, or synthetic estrogens.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

REPORT ON MISCELLANEOUS DRUGS

By I. SCHURMAN (Food and Drug Administration, Federal Security Agency, Chicago 7, Ill.), *Referee*

Iodine.—Mr. Fine submitted for collaborative study a modification of the official method for iodine (*Methods of Analysis*, 6th Ed. 39.202). The results show conclusively the necessity for having present sufficient organic material for complete recovery of iodine. On the basis of the results the present official method should be reworded as described in the Associate Referee's report, adopted as official, first action, and the subject closed.

Calcium, Phosphorus, and Iron in Vitamin Preparations.—Mr. Banes has submitted a report which includes a collaborative study. The results are in excellent agreement and are of an accuracy and precision to warrant that the method with the addendum be adopted as official, first action, and the subject be closed.

Separation of Bromides, Chlorides, and Iodides.—Mr. Stewart made a progress report and recommends that the subject be continued.

Mercury Compounds.—Mr. Green reports that preliminary work with Rotondaro method for small amounts of mercury compounds in creams is not applicable and recommends that the subject be continued.

Methyl Alcohol.—Mr. Guymon was appointed last year as Associate Referee to study the procedures for the determination of methyl alcohol which appear in Sec. 16.25 and Secs. 39.161–2 of the *Methods of Analysis*, 6th Ed., with the view to any needed revision and unification of directions.

Mr. Guymon has submitted a report covering the effect of temperature upon color development and recommends that the study be continued.

RECOMMENDATIONS*

It is recommended—

(1) That the official method for iodine (39.202) be reworded as recommended by the Associate Referee and be adopted as official, final action.

(2) That the method for the assay for calcium, phosphorous, and iron be adopted as official, first action.

(3) That the studies of the separation of bromides, chlorides, and iodides be continued.

(4) That the study of mercury compounds be continued.

(5) That the study of methyl alcohol be continued.

(6) That the following topics on which no reports have been received be continued for another year:

Alkali Metals

Glycols and Related Compounds

Preservatives and Bacteriostatic Agents in Ampul Solutions

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

Microscopic Tests for Alkaloids and Synthetics

Estrone and Estradiol

Chromatographic Separation of Drugs.

REPORT ON ASSAY OF MERCURY COMPOUNDS

By MELVIN W. GREEN (American Pharmaceutical Association Laboratory, Washington, D. C.), *Associate Referee*

At the start of the refereeship on the determination of mercury in phenylmercuric acetate and iodide, it appeared that the portion of the problem needing solution most urgently was the microdetermination of mercury in ointments and creams.

Members of the laboratory staff of the American Pharmaceutical Association had previously determined mercury in all of the official drugs and dosage forms containing mercury by the Rotundaro procedure or some modification of it (1, 2). In this method the mercury is reduced to the metallic state by refluxing with a mixture of ethanolamine, butyric alcohol, and zinc dust. Since the method was essentially satisfactory our attention was immediately focused on an attempt to use the same basic reduction procedure on creams containing less than 1 per cent of phenylmercuric acetate. All attempts to modify such a procedure for small amounts of mercury ended in complete failure.

Due to the impossibility of freeing the small amount of mercury from such a large mass of organic matter, mostly lipoids, attention was turned to the procedure of Laug and Nelson (3), a procedure which frees the mercury by digestion with nitric and sulfuric acids.

After much painstaking work, conditions were found which gave recovery of 97 to 100 per cent of the mercury from such mixtures. Attention was then turned to the preparation of a cream containing small amounts of phenylmercuric acetate homogeneously distributed. This was found to be possible by adding the mercurial in a finely divided state to the molten lipid phase and building the emulsion around it. The emulsified cream had the following formula:

	<i>gram</i>
Phenylmercuric acetate.	0.090
Triethanolamine.	0.110
Glycerin.	3.750
Glycerylmonostearate.	5.250
Stearic acid.	18.000
Water, qs. ad.	150.000

Two creams were prepared according to this formula for the collaborative assays. One (A-2) contained 0.087 g of phenylmercuric acetate per 150 g and the other (B) 0.093 g per 150 g. Both creams were assayed in our laboratory for uniformity by the proposed method to be used by the

collaborators. Cream A-2 yielded a recovery of 98.8 to 99.3 per cent of the known value and Cream B yielded 97.4 to 98.0 per cent of the calculated value.

Since most of the collaborators reported the results in per cent of mercury recovered, this method of reporting will be used in the tabulation of results which are as follows:

TABLE 1.—*Collaborative results*

COLLABORATOR	CALCULATED Hg		RECOVERY OF Hg	
	A2	B	A2	B
	<i>per cent</i>		<i>per cent</i>	
	0.034	0.037		
Collaborator A			0.0338 0.0336	0.0360 0.0362
Collaborator B			0.0313 0.0319	0.0204 0.0218
Collaborator C			0.056 0.058 0.058	0.049 0.048 0.051
Collaborator D			0.060 0.068	0.063 —

COMMENTS BY COLLABORATORS

Collaborator B: Soft glass separatory funnels from our special lead-free stock were used because pyrex funnels were not available.

The curve obtained was a straight line which passed thru every one of 5 points. A Beckman spectrophotometer was used to measure absorption at 490 m μ .

Collaborator C: The directions for addition of hydrogen peroxide until no more brown fumes pass off was somewhat confusing. The brown fumes produced during digestion may be removed with hydrogen peroxide but on heating further more brown fumes form. It may not be necessary to have complete removal of nitrates so I only added hydrogen peroxide for the removal of brown fumes produced during the first part of the digestion. While adding hydrogen peroxide the heat was lowered.

If the sulfuric acid used in preparing standards contains sulfur dioxide this may be destroyed by adding a dilute solution of potassium permanganate until a slight excess remains for a few minutes.

Alcohol used as a preservative for the chloroform should contain no aldehydes.

The mercury dithizonate extracted appears somewhat unstable when put in the spectrophotometer but the lowest per cent T obtainable was used. For 10 micrograms of mercury the readings vary from 57.0 per cent to 61.0 per cent T during three minutes.

Mercury is not completely extracted by the extraction procedure and as larger

amounts of mercury (over 8 micrograms) are present smaller percentage of mercury are extracted. By comparing the procedure with a curve made by extracting mercury from a solution of mercuric sulfate in (1+9) sulfuric acid with dithizone (5.5 mg./liter) 78 per cent of 10 micrograms of mercury and 83 per cent of 8 micrograms of mercury are present. Ag should give negative interference when present in quantities which will ppt. as AgCl. Pb and Bi should give negative interference when present in quantities which will ppt. as sulfates. Copper should offer little interference. Iodine should give negative interference. Bromine may give negative interference.

The method should give reproducible results if the above elements are not present in interfering amounts and if portions of the same extracting solutions are used for both the standards and sample.

Collaborator D: This collaborator is familiar with this method, having used the Laug and Nelson method for mercury, *This Journal*, 25, 399 (1942), on several occasions. It is my opinion that the painstaking precautions and time required cast doubt on the usefulness of this method for an occasional determination of mercury.

Because of the very minute amounts of mercury in the final determination and consequent danger of contamination at any point, the additional precaution of rinsing all glassware with 1+1 HNO₃ followed by water was taken. A Coleman Junior Model 6A Spectrophotometer was used. With the largest size tubes (25 mm. dia.), the 10 ml. of dithizone-mercury extract prescribed was insufficient for obtaining readings. Smaller tubes would have given too narrow a range of readings.

The readings were observed to drift appreciably in the direction of increased transmission very shortly after placing sample in the spectrophotometer, which is contrary to the Laug and Nelson observation that this does not occur with the Coleman instrument. The immediate or lowest reading was the one recorded.

Some adaptation of this method which would permit the determination of large quantities of mercury would also minimize the need for purifying all of the reagents and extreme precautions required, and result in a more practical method. In this connection I call to your attention the mercury method by W. O. Winkler (*Methods of Analysis A.O.A.C.*, 6th ed., p. 470). The final determination by dithizone titration is much simpler than might appear at first reading and I believe more readily applicable to an occasional determination. It seems that adaptation of the HNO₃-H₂SO₄ preparation of sample in the collaborative method to the Winkler determination would offer no great difficulties.

Although many of the results are rather far from the expected results, they are sufficiently uniform to warrant further study. It is recommended* that modified procedures be developed and further samples be assayed in the hope of arriving at an adequate method of assay.

REFERENCES

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- (2) GREEN, N., GREEN, M. W., and POWERS, J. L., *Bull. National Formulary Comm.* 15, 92 (1947).
- (3) LAUG, E. P., and NELSON, K. W., *This Journal*, 25, 399 (1942).

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

REPORT ON SEPARATION OF BROMIDES, CHLORIDES, AND IODIDES

By VINCENT E. STEWART (Food and Drug Laboratory, State of Florida, Department of Agriculture, Tallahassee, Florida), *Associate Referee*

A considerable amount of published and unpublished work on the problem has been done by the previous Associate Referee, N. E. Freeman. The investigation was transferred to this Associate Referee because of the retirement of Mr. Freeman.

Little progress has been made during the year because of the necessity of examining the voluminous literature dealing with the problem. The laboratory investigations were confined largely to a study of the method recommended by Freeman¹ for the determination of chloride in the presence of large amounts of bromide and/or iodide.

This method has been investigated by the Associate Referee and a few samples were submitted to collaborators.

Some of the collaborators were unable to complete the analyses in the short time which was allowed and the results are too incomplete to justify a report at this time. It is obvious that still further investigation is necessary before the procedure can be recommended as a tentative method.

RECOMMENDATIONS*

It is recommended—

(1) That the revised acetone method for the determination of chloride in the presence of large amounts of bromide and/or iodide be investigated further and then subjected to additional collaborative study.

(2) That the volumetric cyanide method for the determination of iodide and bromide in the presence of chloride¹ be compared with the aeration absorption method and that these methods be submitted to collaborators.

REPORT ON IODINE

By SAM D. FINE (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

Last year a preliminary report was made on the need for revision of the present official method for iodine. To summarize that report briefly, it was found that the low results obtained on samples containing little or no organic material could be corrected by the addition of organic material before charring, as directed in the present method. It was recommended that the proposed modification be subjected to collaborative study.

The Associate Referee found it impossible to prepare a mixture of

¹ Freeman, N. E., and Beulah V. McMullen, *This Journal*, 31, 550 (1948).

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 49 (1949).

iodide and other salts which was homogeneous. Accordingly, a mixture of salts of iron, magnesium, sodium, and calcium was prepared and a solution of potassium iodide, stabilized by the addition of alkali, was prepared separately. Collaborators were sent the mixture of inorganic salts and the solution of potassium iodide and asked to add identical aliquots of the

TABLE 1.—*Comparative results*

COLLABORATOR	METHOD 39.202		MODIFICATION OF 39.202	
	MG KI/10 ML	REC.	MG KI/10 ML	REC.
1	33.8	<i>per cent</i> 67.6	49.6	<i>per cent</i> 99.2
	34.8	69.6	49.8	99.6
2	25.3	50.6	49.5	99.0
	41.5	83.0	49.9	99.8
	—	—	49.8	99.6
3	41.8	83.6	49.9	99.8
	41.2	82.4	49.8	99.6
4	33.0	66.0	50.0	100.0
	34.0	68.0	50.0	100.0
5	35.7	71.4	49.7	99.4
	35.7	71.4	49.6	99.2
6	35.4	70.8	50.2	100.4
	36.0	72.0	50.3	100.6
7	35.3	70.6	49.6	99.2
	36.5	73.0	49.8	99.6
Ave.	35.7	71.4	49.8	99.6

iodide solution to identical weights of the inorganic mixture. It was directed that determinations be made in duplicate by the present method and by a modification which consisted essentially in the addition of one gram of starch before the addition of the solid potassium hydroxide. A further modification consisted of the use of 1% ammonium chloride as a wash in place of the water specified by the present method.

The results obtained by the collaborators are shown in Table 1.

DISCUSSION

The Associate Referee noted trouble occasionally with colloidal manganese dioxide running through the filter paper. In such instances the filtrate was returned to the steam bath and allowed to digest until the

manganese dioxide had become flocculent and then a second filtration was made. Rupert Hyatt, of Cincinnati Station, suggested the use of 1% ammonium chloride as a wash and this suggestion was incorporated in the instructions to all collaborators after it had been tried by the Associate Referee and found beneficial in most instances. D. M. Taylor, of the Denver Station, made the following suggestion:

"I have found, I believe, a wash water that works much better than the ammonium chloride solution that you have recommended. This wash water is prepared by running a blank on all reagents in the usual manner and then adding an equal volume of water to the filtered blank. In the nine or ten determinations that I have made in the last year, I have not had any trace of manganese dioxide washing thru into the final solution using this procedure."

The occasions when manganese dioxide have been noted to pass through the filter paper are so seldom that it is felt scarcely necessary by the Associate Referee to adopt Mr. Taylor's suggestion. Redigestion has been found in every instance to produce a clear filtrate and this is believed preferable to the preparation of the wash solution suggested by Taylor.

One of the collaborators at the Cincinnati Station obtained low results on his first set of determinations by the proposed modification. He was asked to repeat the determinations and all steps were closely observed by the Associate Referee. The difficulty was readily apparent in that insufficient permanganate was added to completely oxidize the iodide to iodate. The present method directs:

" . . . Heat to boiling and add saturated KMnO_4 soln slowly until KMnO_4 color remains after several minutes boiling. Then add ca 0.5 ml in excess, continue boiling ca 5 min., and allow to cool. . . . "

The collaborator had failed to add sufficient permanganate to maintain the characteristic coloration for the period specified. A collaborator at another laboratory had the same difficulty. After directing his attention to the necessity of adding permanganate sufficient to maintain the characteristic coloration for the period specified, excellent results were obtained. The same collaborator pointed out that there are no specific instructions for thoroughly mixing the potassium hydroxide with the sample and suggested the use of a stirring rod, allowing it to remain in the crucible during the charring.

The possibilities of failure to mix the alkali thoroughly with the iodide and of failure to add sufficient permanganate to oxidize completely to iodate had not occurred to the Associate Referee. However, the collaborators' comments and results indicate a definite need for cautionary statements relative to these two steps in the procedure.

REWORDING OF THE OFFICIAL METHOD

The rewording of the official method is intended to incorporate the modification previously suggested for samples low in organic material and

also the cautionary statements that are indicated as a result of collaborative study. The details of the method as modified are given in *This Journal*, 32, 115 (1949).

ACKNOWLEDGMENT

Acknowledgment is gratefully extended to the following collaborators from the Food and Drug Administration who participated in this study: D. M. Taylor, Denver Station; C. R. Joiner, St. Louis Station; Sidney Williams, Rupert Hyatt, F. J. McNall, and H. C. Van Dame, Cincinnati Station.

It is recommended*—

That the present official method for iodine be reworded as described in this report.

REPORT ON CALCIUM, PHOSPHORUS, AND IRON IN VITAMIN PREPARATIONS

By DANIEL BANES (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Calcium, phosphorus, and iron are among the elements indispensable for normal metabolism, and they are included in many special dietary and vitamin preparations. There are already present in the A.O.A.C. *Methods of Analysis* several excellent methods describing the assay of organic materials for these substances. This study was therefore limited to selecting that procedure deemed most readily applicable to vitamin preparations.

The volumetric method for calcium as the oxalate, employing potassium permanganate solution, is rapid and accurate, even in the presence of phosphorus, iron, aluminum, and magnesium (1). Phosphorus in the orthophosphate form is easily estimated by alkaline titration of precipitated ammonium phosphomolybdate. (Pyrophosphates, to which the method is inapplicable, are often encountered, but they are quantitatively converted to the ortho- state by heating with concentrated hydrochloric acid.) The colorimetric method for iron using α - α dipyridyl or *o*-phenanthroline requires only small amounts of the metal, and it is reliable. All three procedures are applicable to dilute acid solutions of ashed materials.

Significant losses of iron during dry ashing have been reported (2, 3), presumably due to the volatilization of ferric chloride. To test the extent of this loss a series of powdered samples (a-d), each containing 5 mg of iron were analyzed. (a) was mixed with 5 ml of normal sodium hydroxide; (b) was mixed with 5 ml of normal sodium chloride; (c) was mixed with 3 ml of normal sodium chloride and 2 ml of concentrated hydrochloric acid; (d) was untreated. The four mixtures after drying and ashing

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 80 (1949).

yielded congruous results within the limits of error, indicating that the volatilization of iron is negligible in quantities of the order of 1-10 mg of iron per sample. Munsey (4) previously reported similar data for cereal products.

RESULTS OF ANALYSIS

Two samples were prepared for collaborative study. Sample A was a commercial vitamin product consisting of tablets which were ground together and passed through a 150-mesh sieve four times. Sample B was a synthetic mixture prepared from purified starch, standard ferrous ammonium sulfate, thrice recrystallized potassium acid phosphate, and calcium carbonate derived from precipitated calcium oxalate. It contained 35.8 mg of calcium, 10.9 mg of phosphorus, and 8.16 mg of iron per gram.* The results are shown in Table 1.

TABLE 1.—Calcium, phosphorus, and iron in vitamin preparations

COLLABORATOR	SAMPLE A			SAMPLE B		
	Ca	P	Fe	Ca	P	Fe
	mg/gm	mg/gm	mg/gm	mg/gm	mg/gm	mg/gm
H. F. O'Keefe	124.3	96.7	8.7	35.1	10.7	8.2
	124.1	98.0	8.7	35.8	10.9	8.2
D. Banes	122.5	97.8	8.7	35.3	10.8	8.0
	124.6	97.2	8.7	35.3	10.8	8.0
R. Hyatt	122.4	96.1	8.7	35.1	11.1	8.5
	122.9	96.0	8.7	35.4	11.1	8.3
S. D. Fine	123.3	97.4	—	35.9	11.2	—
	123.5	97.2	—	35.9	11.2	—
G. E. Keppel	122.3	94.2	8.5	35.2	10.6	8.5
	122.6	94.5	8.5	34.8	10.7	8.7
H. R. Bond	120.8	96.9	8.8	34.4	10.7	8.0
	121.6	97.3	9.0	34.7	10.9	8.3
Average	122.9	96.6	8.7	35.3	10.9	8.2
Average Recovery %	—	—	—	98.6	100.0	101.3

COMMENTS OF COLLABORATORS

Rupert Hyatt.—“The method should be broadened to accommodate analyses for other elements, such as magnesium. This could be accomplished by adding at the end of the calcium method: . . . Reserve the filtrate for the determination of magnesium as in *Methods of Analysis*, 1945, 26.21-4.”

* Details of the method are given in *This Journal*, 32, 114 (1949).

DISCUSSION AND RECOMMENDATION

The data reported indicate that the proposed methods are of an accuracy and precision suitable for the analysis of vitamin preparations. It is recommended* that the method, with the addendum suggested, be adopted as official, first action, and that the subject be closed.

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- (2) HOFFMAN, C., SCHWEITZER, T. R., and DALBY, G., *Ind. Eng. Chem., Anal. Ed.*, **12**, 454 (1940).
- (3) GRAY, P. P., and STONE, I. M., *ibid.*, **10**, 415 (1948).
- (4) MUNSEY, V. E., *This Journal*, **27**, 398 (1944).

No reports were given on microscopic tests for alkaloids and synthetics, alkali metals, glycols and related compounds, preservatives and bacteriostatic agents in ampul solutions, estrone and estradiol, or chromatographic separation of drugs.

For report of methyl alcohol, see "Methanol in Distilled Spirits," by J. F. Guymon, *This Journal*, **32**, 163 (1949).

* For report of Subcommittee B and action of the Association, see *This Journal*, **32**, 50 (1949)

TUESDAY—AFTERNOON SESSION

REPORT ON STANDARDIZATION OF MICROCHEMICAL METHODS. CARBON, HYDROGEN, AND NITROGEN

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This year's studies, following the recommendations set forth in last year's report, have been devoted to the determination of carbon and hydrogen and nitrogen by the Kjeldahl and Dumas procedures. The selection was based on the results of a questionnaire, which indicated that in the opinion of a majority of micro analysts these determinations should be studied first.

Two samples, nicotinic acid and benzyl-iso-thiourea hydrochloride, were sent to two groups of collaborators. One group was asked to analyze the samples for carbon and hydrogen; the other was asked to determine nitrogen both by the Kjeldahl and Dumas procedures. These compounds were chosen for this work because both are stable and nonhygroscopic and because they differ considerably in constitution and ease of decomposition.

Although a statement of purity did not accompany the samples, the collaborators were informed that they were relatively pure. They were asked to report all the numerical values obtained for each sample, regardless of whether or not the data appeared to be correct.

The collaborators for carbon and hydrogen were requested to analyze the two samples by their own methods only, since there is no one method in common use today. The collaborators for nitrogen were asked to analyze the two samples by their own Kjeldahl and Dumas methods and by the A.O.A.C. Tentative Microkjeldahl Method.

Questionnaires accompanying the samples asked for details of the apparatus, procedure, and laboratory conditions under which the analyses were conducted. From the information so obtained and the accompanying analytical results, a method will be adopted or devised for each determination, and each will be given rigorous collaborative study before it is proposed as a tentative method. Since so many methods were used in obtaining the data for each determination and consequently the number of variables was so large, the data have been analyzed statistically in an attempt to determine which variations in procedure and apparatus are important. In the statistical comparisons of these data influenced by a large number of variables, the assumption was made that all variables other than the one being evaluated cancelled themselves. The conclusions drawn are based on the available data, and although there is a possibility

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that they may be erroneous because of the assumption on which they are based, they will be used as guides for future studies until more data are available. Since some data were received after the analysis of the data was started, the total number of analyses is in some cases greater than that used in the statistical treatment.

CARBON AND HYDROGEN DETERMINATION

Twenty-five analysts from 19 laboratories reported 111 carbon and hydrogen analyses for sample 1 (nicotinic acid) and 92 analyses for

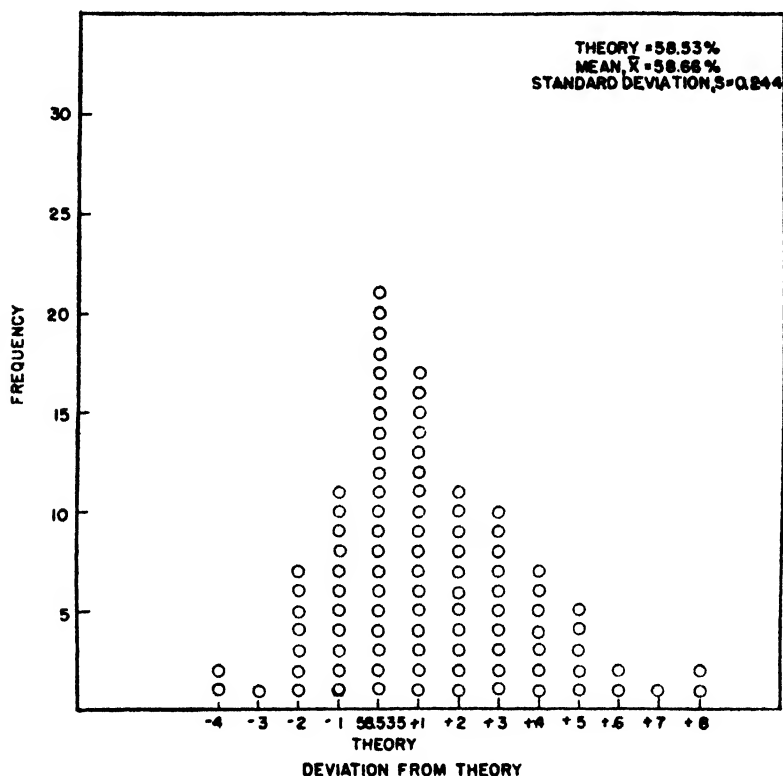


Fig. 1.—Histogram of carbon values for nicotinic acid.

sample 2 (benzyl-iso-thiourea hydrochloride). The number of analyses reported from different laboratories ranged from 2 to 20.

Carbon.—The histogram (Fig. 1) shows the frequency distribution of the carbon values obtained for nicotinic acid. Although all points in the figure are located on the class marks, they show the number of analyses which fell within the class boundaries (class mark $\pm 0.05\%$). Three results are not shown and are not included in the subsequent treatment. The chi square distribution test for measuring goodness of fit of frequency distribution showed that these three values fell outside the representative

population of the carbon values for this compound. This test has shown that the values retained in the histogram are a representative sample of a normal population, and therefore they can be treated statistically.

Figure 1 shows that a majority of the values are higher than the theoretical value (58.53%). The mean (\bar{X} or average) of the values is 58.66%, and the standard deviation (S, the variation about a mean) is 0.244, indicating that 67% of the carbon values for this sample should fall within ± 0.244 or 95% within ± 0.488 of the mean value.

Although the mean is 0.13% higher than the theoretical value, the mode (class which contains the largest number of values) falls on theory, indicating that there is a good possibility of finding among the methods one which will give high accuracy as well as good precision. Inspection shows that even though there is a preponderance of values above the theoretical value, the skewness of the histogram toward the theoretical value and the mode indicate that there is a tendency to obtain theoretical values.

All carbon methods followed the same general procedure, which consisted in the catalytic combustion of a weighed sample to carbon dioxide, followed by the absorption and weighing of the combustion product. Although no two methods were identical, each step in any one method was in general similar to the corresponding step in several other methods. Therefore, the carbon values for nicotinic acid were divided into two groups, one representing the results obtained by a certain operation for one step in the determination, and the other a second operation for the same step. These two groups were treated statistically to determine whether or not there was a significant difference in the results obtained by the two operations for the step or condition in question. By this procedure, the following comparisons were made: (1) electrical *vs.* gas sample burner, (2) mechanical *vs.* hand-operated sample burner, (3) semimicro (10–30 mg) *vs.* micro (2–10 mg) sample weight, (4) air-conditioned *vs.* nonair-conditioned laboratories, (5) balance in an air-conditioned balance room *vs.* balance adjacent to the furnace. The only case in which there was a significant difference in the carbon results for nicotinic acid was semimicro *vs.* micro samples. The significance level used throughout this study was 5%, which means that if the experiment was repeated a number of times the differences obtained should be at least as large as the one found in 95% of the cases. The theoretical frequency distribution curves for these two methods are shown in Figure 2. The means (\bar{X}) for the two procedures were 58.52 and 58.69, whereas the standard deviations (S) were 0.198 and 0.239, respectively. Since the difference in the two means was significant, and the semimicro mean was closer to the theoretical value, better values can be obtained by the semimicro than the micro method and, similarly, since the standard deviation is less, the precision of the semimicro method is greater.

The data for sample 2, benzyl-iso-thiourea hydrochloride, were treated in the same manner as those for nicotinic acid. Figure 3 shows the histogram of the values obtained for this material. The chi square distribution test showed that the values obtained should all be included in the

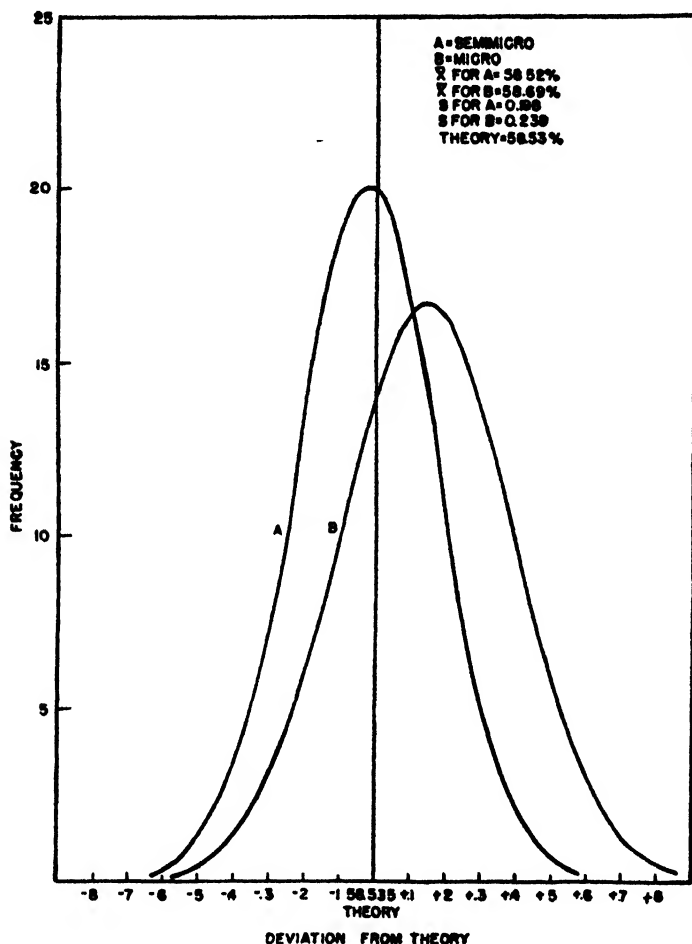


FIG. 2.—Theoretical distribution curves for micro and semimicro carbon values. Nicotinic acid.

statistical treatment. The mean (\bar{X}) of the values is 47.51% or 0.11% above theory, with a standard deviation (S) of 0.184. Since the mean is nearer the theoretical value for this sample than that for sample 1 (nicotinic acid), and the standard deviations is less, the methods used gave slightly better carbon values for sample 2. These differences must be due to an inherent difference in the two compounds, since the methods and conditions were the same for the analyses of the two samples. The same comparisons were made for the values for benzyl-iso-thiourea hydro-

chloride as were made for nicotinic acid. There is again a significant difference between the values for the semimicro *vs.* micro procedures and, as for sample 1, the difference is in favor of the semimicro method. Figure 4 shows the theoretical frequency distribution curves for the two methods. The means are 47.38% and 47.53%, and the standard deviations 0.078 and 0.189 for the semimicro and micro methods, respectively.

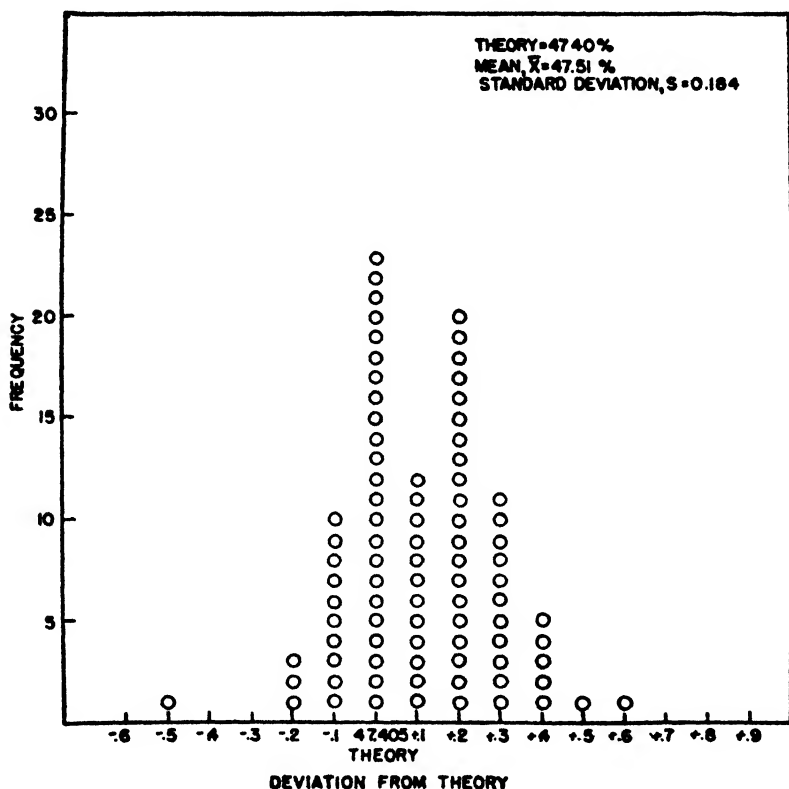


FIG. 3.—Histogram of carbon values for benzyl-iso-thiourea hydrochloride.

The results of the carbon analyses for the two samples are similar, in that the mode in both cases falls on theory, the means are above theory, and the standard deviations for the two are comparable. Semimicro methods were significantly better than micro methods for both samples, as is shown in Figures 2 and 4. None of the other comparisons showed any significant difference which would lead one to recommend the preferential use or adaptation of one procedure over another. Table 1, however, shows slight trends which favor certain operations, and these may serve as guides in planning future studies.

Hydrogen.—The number of hydrogen values received and the number of

collaborators were the same as for carbon, since the two determinations were made simultaneously. Histograms of the hydrogen values for nicotinic acid and benzyl-iso-thiourea hydrochloride are shown in Figures 5 and 6, respectively.

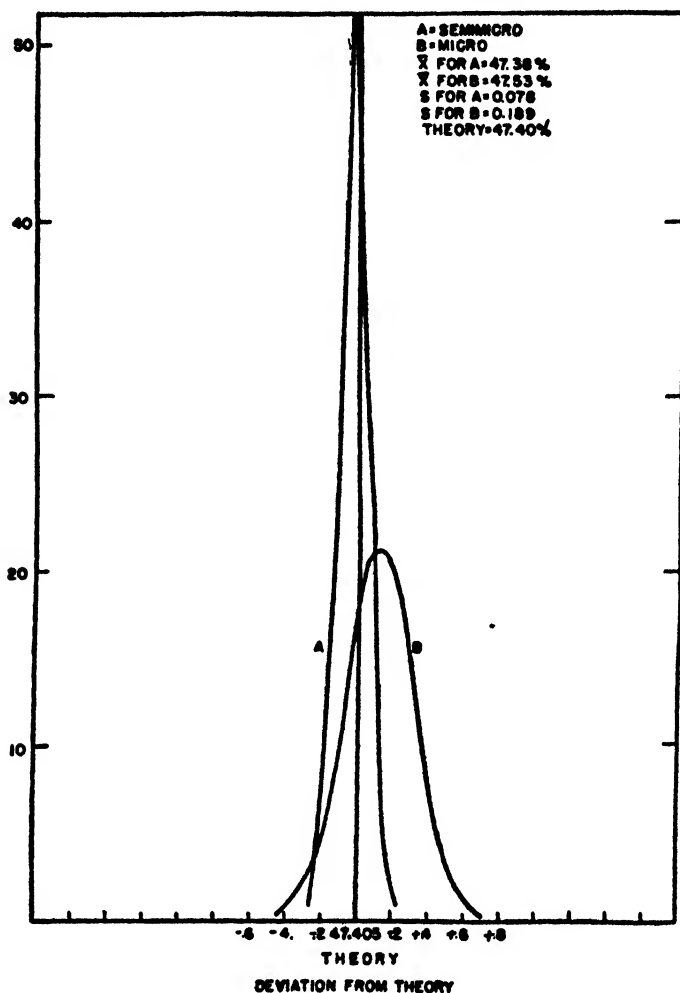


FIG. 4.—Theoretical frequency distribution curves for semimicro and micro carbon values. Benzyl-iso-thiourea hydrochloride.

A marked similarity in the data for the two samples can be seen. The means are 0.10 and 0.06% above the theoretical value, and the two standard deviations are 0.208 and 0.200. In both cases the mode, class with the highest frequency, is 0.10% above theory.

The same operations as listed previously for carbon were compared to determine whether or not they were significantly different. The values

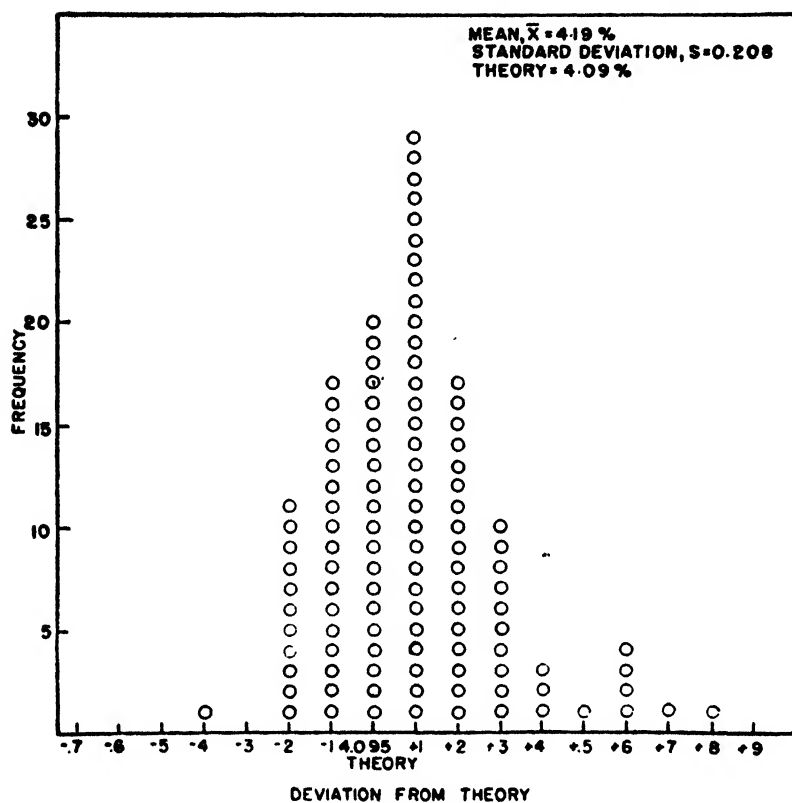


FIG. 5.—Histogram of the hydrogen values for nicotinic acid.

TABLE 1.—Carbon data obtained for samples 1 and 2 by catalytic combustion methods

	NICOTINIC ACID (1)			BENZYL-ISO-THIOUREA HYDROCHLORIDE (2)		
	NUMBER OF SAMPLES	\bar{X}	S	NUMBER OF SAMPLES	\bar{X}	S
		per cent			per cent	
Semimicro method	16	58.52	0.198	14	47.38	0.078
Micro method	81	58.69	0.239	76	47.53	0.189
Electric sample burner	50	58.70	0.268	45	47.51	0.148
Gas burner	47	58.62	0.205	44	47.51	0.195
Mechanical burner	41	58.67	0.276	41	47.49	0.143
Hand-operated sample burner	56	58.65	0.209	49	47.53	0.212
Balance in air-conditioned balance room	42	58.72	0.286	34	47.54	0.161
Balance adjacent to furnace	34	58.61	0.221	43	47.47	0.200
Air-conditioned laboratory	59	58.65	0.241	52	47.51	0.176
Non-air-conditioned laboratory	38	58.67	0.179	38	47.52	0.200
Total samples	97	58.66	0.244	90	47.51	0.184
Theoretical values		58.53			47.40	

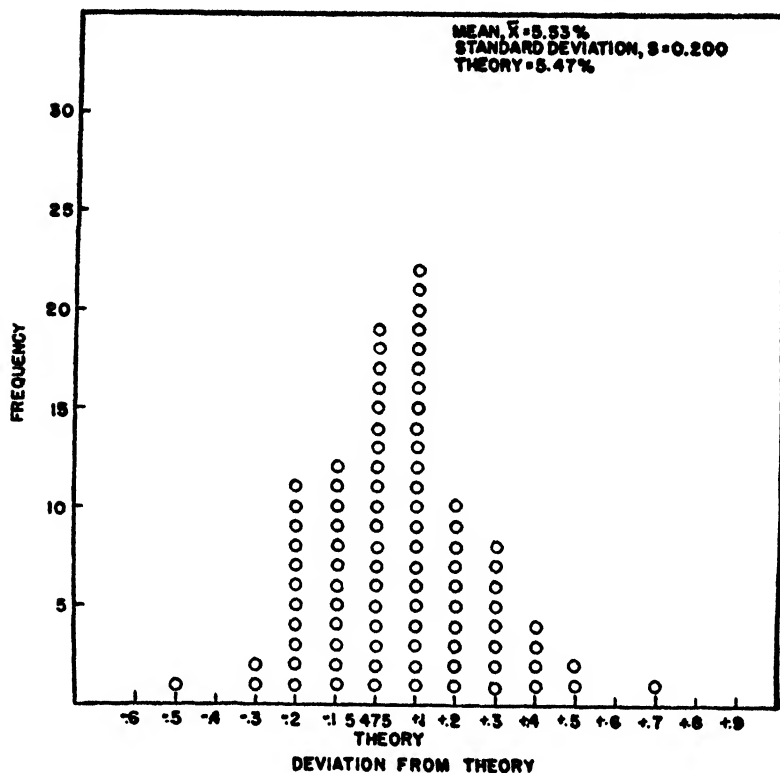


FIG. 6.—Histogram of hydrogen values for benzyl-iso-thiourea hydrochloride.

TABLE 2.—Hydrogen data obtained for samples 1 and 2 by catalytic combustion methods

	NICOTINIC ACID (1)			BENZYL-ISO-THIOUREA HYDROCHLORIDE (2)		
	NUMBER OF SAMPLES	\bar{X}	S	NUMBER OF SAMPLES	\bar{X}	S
		per cent			per cent	
Semimicro method	18	4.20	0.161	12	5.57	0.109
Micro method	87	4.21	0.214	80	5.53	0.207
Electrical burner	63	4.15	0.175	47	5.51	0.190
Gas burner	52	4.23	0.235	45	5.56	0.205
Mechanical burner	58	4.14	0.195	43	5.48	0.179
Hand-operated burner	57	4.24	0.229	48	5.58	0.210
Balance in air-conditioned balance room	41	4.17	0.176	34	5.50	0.207
Balance adjacent to furnace	44	4.22	0.261	40	5.55	0.219
Air-conditioned	62	4.16	0.178	53	5.49	0.182
Non-air-conditioned laboratory	53	4.23	0.237	39	5.59	0.205
Total samples	115	4.19	0.208	92	5.53	0.200
Theoretical values		4.09			5.47	

shown in Table 2 for mechanically operated sample burners are significantly better than those for hand-operated burners. Figures 7 and 8 show the theoretical frequency distribution curves for mechanical *vs.* hand-operated furnaces for samples 1 and 2, respectively. Only one other comparison, air-conditioning *vs.* non-air-conditioning, proved to be significant. The theoretical frequency distributions in Figure 9 show that

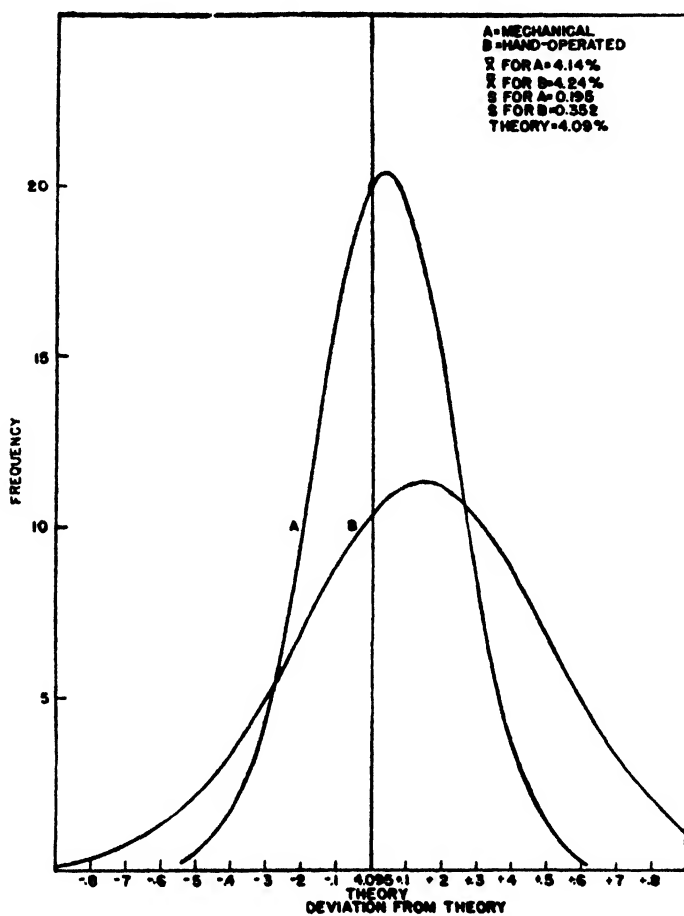


FIG. 7.—Theoretical frequency distribution curves for hydrogen values from mechanical and hand-operated furnaces. Nicotinic acid.

air-conditioned laboratories were superior for the hydrogen analysis of benzyl-iso-thiourea hydrochloride. While the mean is nearer theory and the S value smaller for hydrogen values from air-conditioned laboratories for nicotinic acid, the difference was not significant.

Summary for carbon and hydrogen.—A comparison of the data for hydrogen with those for carbon shows that the means, \bar{X} , for hydrogen

are slightly closer to theory than those for carbon, that in both analyses the means are above theory, and that standard deviations or precisions for hydrogen and carbon for both samples are similar.

The means for the carbon and hydrogen values obtained in air-conditioned laboratories are closer to the theoretical value than those

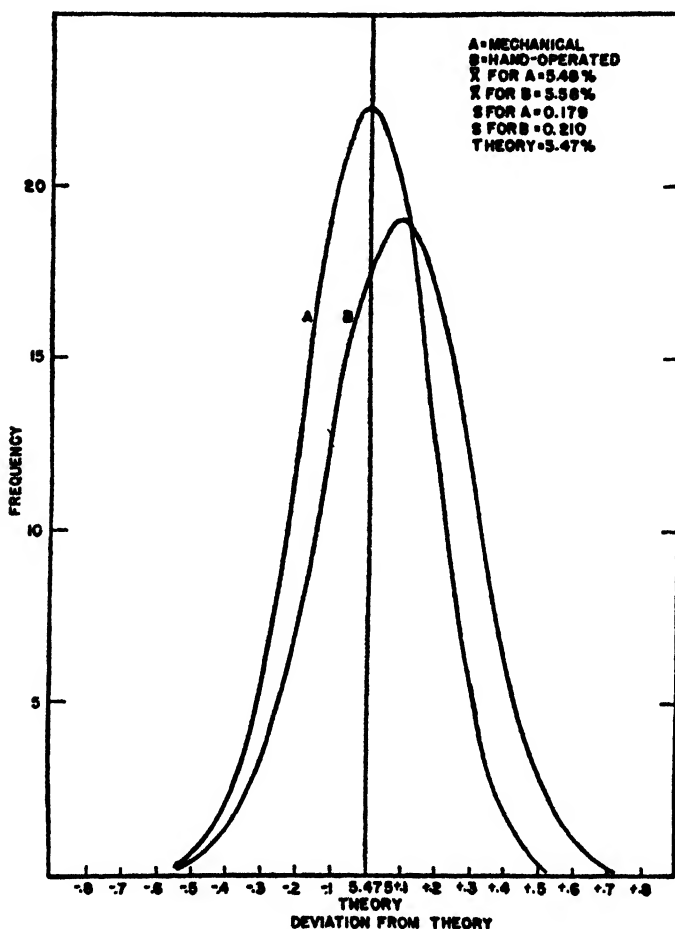


FIG. 8.—Theoretical frequency distribution curves for hydrogen values from mechanical and hand-operated furnaces. Benzyl-iso-thiourea hydrochloride.

made in non-air-conditioned laboratories, but the difference is significant only in the hydrogen values of sample 2. In three of the four determinations, the standard deviation (S) is lower for air-conditioned laboratories.

Comparison of the results obtained by mechanical *vs.* hand-operated furnaces showed that in three of the four determinations, mechanically operated furnaces gave means, \bar{X} , closer to the theoretical value and in two cases the difference was significant. In general, the means for electrical

burning furnaces are nearer the theoretical value than those for gas burners, and the standard deviations are less, but in no case is the difference significant.

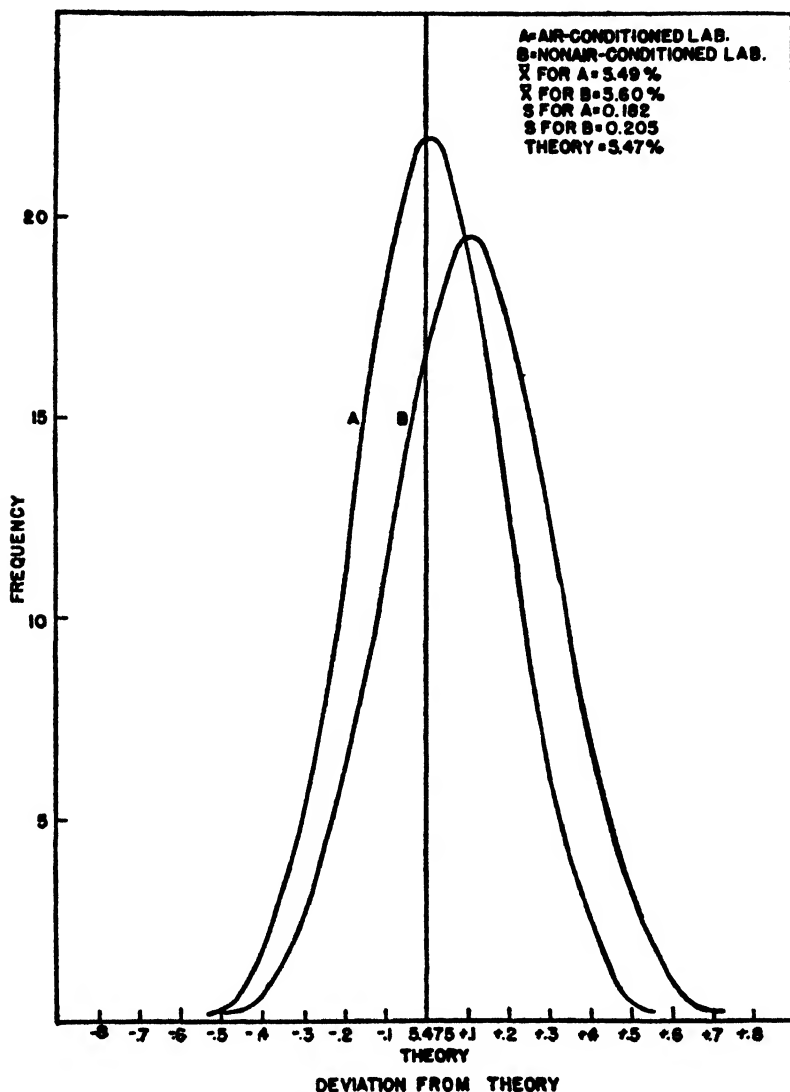


FIG. 9.—Theoretical frequency distribution curves for hydrogen values from air-conditioned and non-air-conditioned laboratories. Benzyl-iso-thiourea hydrochloride.

Perhaps the most significant comparison is that of the values obtained by semimicro and micro procedures for carbon. Here the semimicro procedures are superior, provided that the variables other than size of sample

can be neglected. As for the hydrogen values, the standard deviation also favors the semimicro method, and the average values are nearly identical.

Comparison of values obtained when the balance was in an air-conditioned balance room *vs.* those obtained when the balance was adjacent to the furnace shows that the means are nearer the theoretical value and the standard deviations are lower for carbon values when the latter method

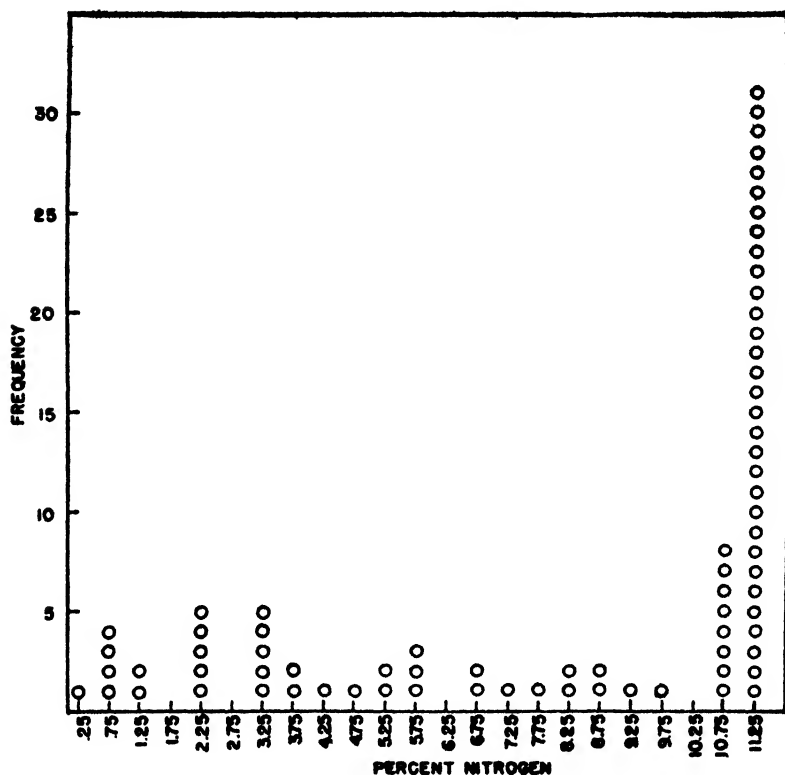


FIG. 10.—Histogram of Kjeldahl nitrogen values for nicotinic acid. Theory = 11.38.

was used, but the reverse is true for the hydrogen values. In no case, however, is the difference between the two procedures statistically significant. More comparisons of the different phases of the analysis, including tube fillings, absorbents, rate of gas flow and the like, are in progress and must be completed before a trial method can be recommended.

KJELDAHL NITROGEN DETERMINATION

The same two compounds used in the carbon and hydrogen studies were sent to a second group of collaborators, who were asked to determine the nitrogen in the two samples by both the Kjeldahl and Dumas procedures.

They were also asked to make the Kjeldahl analyses by their own method and by the A.O.A.C. (tentative) Microkjeldahl Method, a copy of which was enclosed.

One of the reasons for choosing nicotinic acid was that it contains a ring nitrogen, which is difficult to obtain by the Kjeldahl method. Replies to the questionnaire which accompanied the samples indicated that some

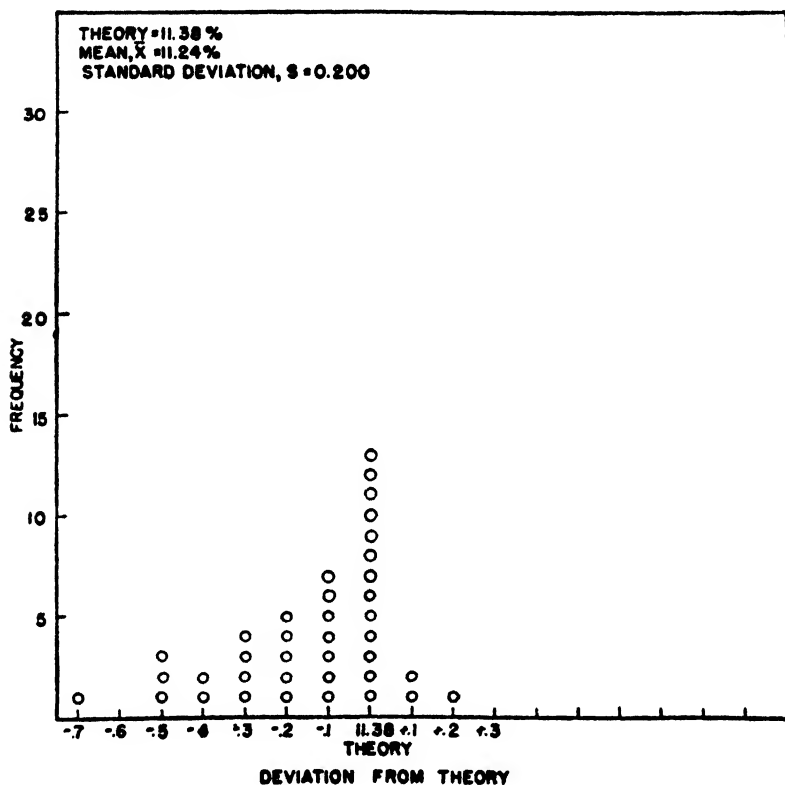


FIG. 11. Histogram of the Kjeldahl nitrogen values for nicotinic acid from the two upper classes in Fig. 10.

micro analysts do not attempt to determine nitrogen in such compounds by the Kjeldahl method but others do it regularly. Therefore, the nitrogen values obtained by the A.O.A.C. method should be a good test of its reliability.

The histogram of the population of the 75 values from twelve collaborators is shown in Figure 10. To present all the data in one histogram, the class intervals had to be in units of 0.5%, since the reported nitrogen values ranged from less than 0.5 to 11.5%.

It is obvious from the histogram that the data as a whole can not be treated statistically but that there is a sharp division of the values, about half being near the theoretical value and the remainder low and erratic.

The collaborators who reported results from 0 to 10% obtained no values higher than 11%, whereas those who reported values about 11% had no values below 10.5%. A histogram (Fig. 11) with class limits of 0.1% was made of the data obtained by those methods which gave one or more values above 11%. Although the mode falls on the theoretical value,

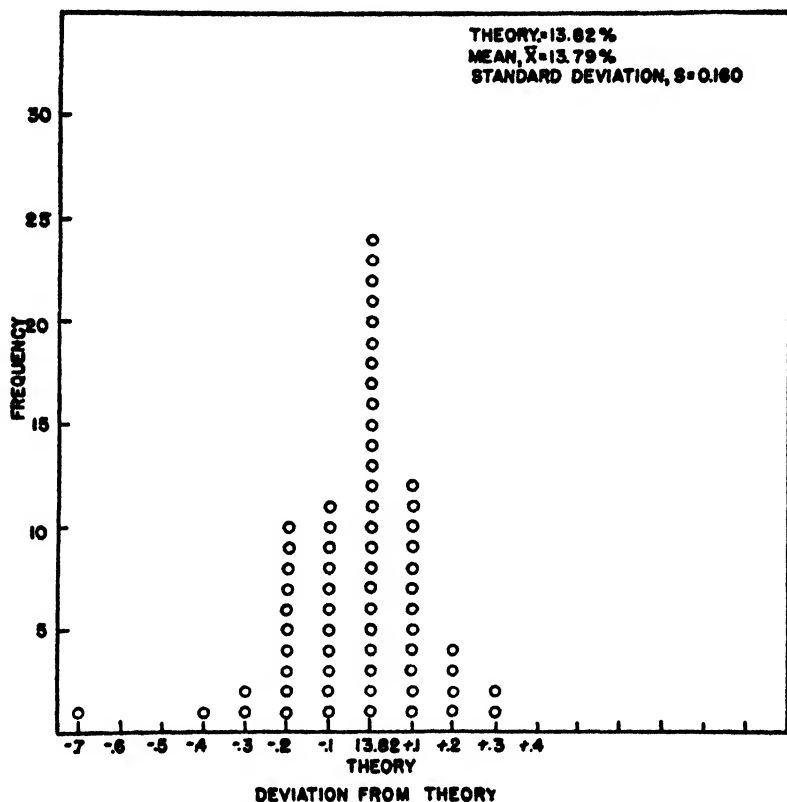


FIG. 12.—Histogram of Kjeldahl nitrogen values for benzyl-isothiourea hydrochloride.

the mean is considerably below this value, indicating much skewness in the data. The chi square test showed that the data are not a representative sample of a normal population, and therefore statistical comparisons can not be made. Nevertheless, the means were determined for those values obtained when mercury and mercury plus selenium were used as catalysts, and the mean was closer to the theoretical value when only mercury was used. The many values which are in agreement with the theoretical value indicate that a satisfactory method can be found.

The analysis of benzyl-isothiourea hydrochloride (sample 2) for nitrogen by the Kjeldahl method proved to be a much simpler task than

determining the nitrogen in nicotinic acid. Figure 12, the histogram of the values, shows that there is a fairly normal distribution of the values around the mode which falls on the theoretical value. The chi square distribution test indicated that the 67 values received are a representative sample and all should be included in the studies. The mean is 13.79, only 0.03% less than theory, and the standard deviation is 0.160.

TABLE 3.—*Nitrogen data obtained for sample 2 by the Kjeldahl method*

	BENZYL-ISO-THIOUREA HYDROCHLORIDE		
	NUMBER OF SAMPLES	\bar{X}	S
		<i>per cent</i>	
Semimicro methods	18	13.76	0.212
Micro methods	49	13.81	0.134
Mixed indicator	31	13.77	0.190
Single indicator	36	13.81	0.126
Digestion aid	35	13.80	0.118
No digestion aid	32	13.78	0.195
Electrical digestion	11	13.75	0.224
Gas digestion	56	13.80	0.141
Mercury catalyst	45	13.77	0.167
Mercury plus selenium catalyst	22	13.83	0.134
Parnas-Wargner apparatus	33	13.76	0.167
Other distillation apparatus	34	13.83	0.148
One-half hour digestion	10	13.73	0.228
One hour digestion	57	13.80	0.145
Silver or tin condenser tubes	34	13.75	0.170
Pyrex condenser tubes	33	13.84	0.134
Total samples	67	13.79	0.160
Theoretical values		13.82	

Twenty-three of the values were obtained by the A.O.A.C. procedure, and their mean is 13.795 with a standard deviation of 0.122. The remaining 44 values by various micro and semimicro procedures have a mean of 13.791 with a standard deviation of 0.176. The difference between the means is far from significant, but slightly better precision (smaller S value), was obtained with the A.O.A.C. procedure than with the sum of the other methods.

The data were used to make 8 other comparisons of variations in the Kjeldahl procedure. The comparisons and the mean and standard deviation of each are shown in Table 3. There was no significant difference between the means for any of the comparisons except for silver or tin versus Pyrex glass condenser tubes, the Pyrex glass being favored. The difference between the means for one-half and one hour digestions was nearly significant and in favor of the longer digestion.

The difference between the means for the Parnas-Wagner and other distillation apparatus is considerable, but there is so much duplication of the values obtained by this apparatus and the silver or tin condenser that without further study it is not possible to say whether the lower values are due to the apparatus, the condenser, or both.

Summary for Kjeldahl nitrogen.—The data for nicotinic acid indicate that the A.O.A.C. (tentative) Microkjeldahl method is unsatisfactory for many compounds with ring nitrogen, but it does show that a satisfactory method can probably be established, since four collaborators using four different methods obtained values in agreement with the theoretical values. For the less refractory material, sample 2, the A.O.A.C. method gave values with an excellent mean and with better precision than the sum of the other Kjeldahl methods used. The results indicate that the A.O.A.C. method will apply to compounds with ring nitrogen if the catalyst and digestion time are both increased by amounts yet to be determined.

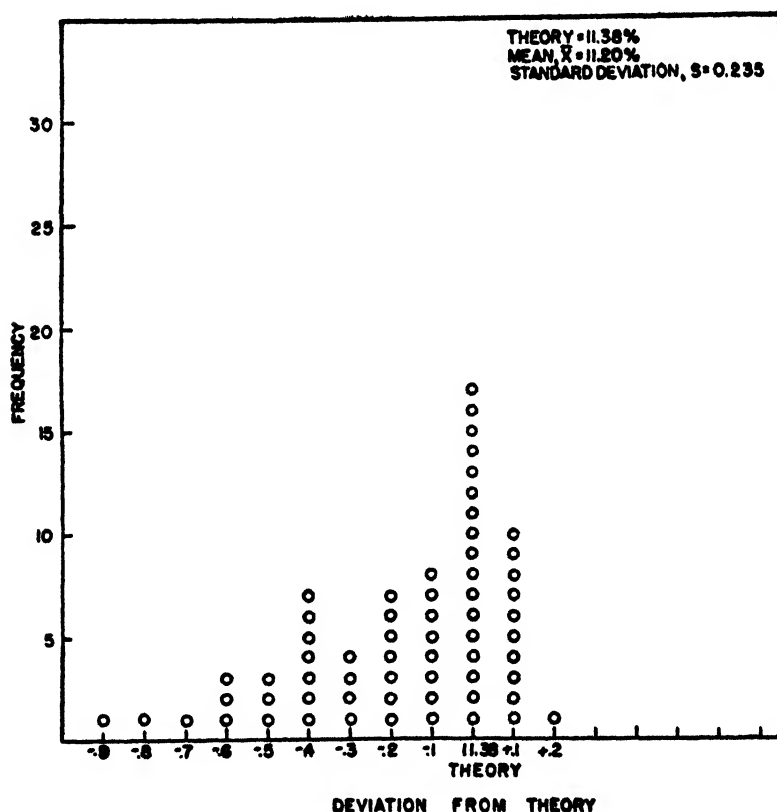


FIG. 13.—Histogram of Dumas nitrogen values for nicotinic acid.

DUMAS NITROGEN DETERMINATION

The same collaborators participated in this determination as in the Kjeldahl studies, and they used the same two samples. Histograms of the values obtained by the 13 collaborators for samples 1 and 2 are shown in Figures 13 and 14, respectively. The values for neither compound were representative samples of normal populations, as shown by the chi square test. Inspection of the histograms shows too many values in the classes

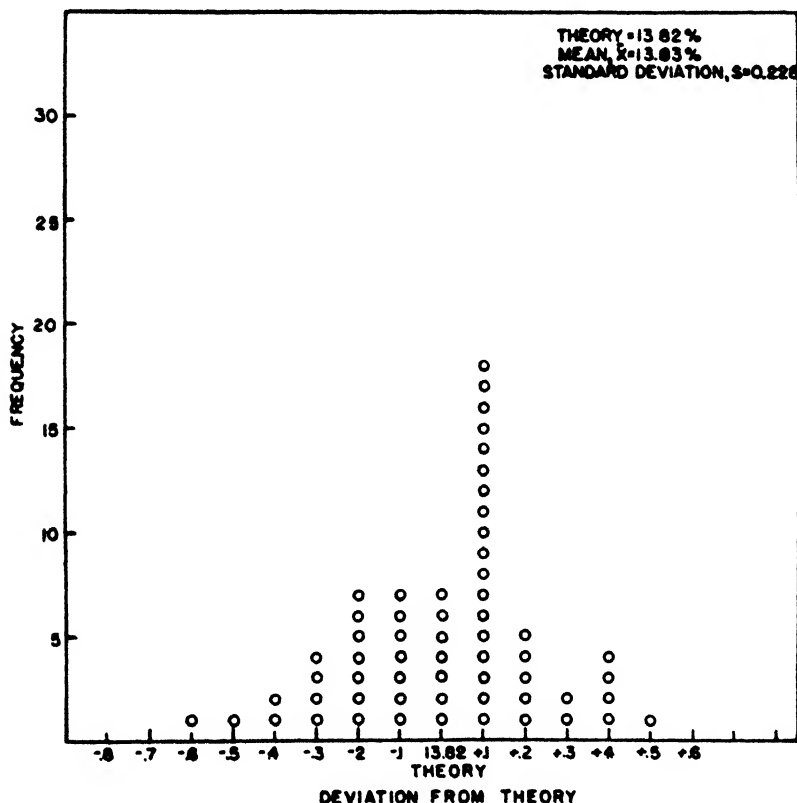


FIG. 14.—Histogram of Dumas nitrogen values for benzyl-iso-thiourea hydrochloride.

below theory, and nicotinic acid has a second mode 0.4% below the theoretical value. The questionnaires indicated that most of the lower values were obtained by methods which used temperatures below 650° C. Consequently, the nitrogen values for nicotinic acid obtained by methods with temperatures above 650°C. were tested and found to be representative of a normal population. Although the data for sample 2 were not as skewed as for sample 1, they were treated in the same manner and with the same result.

The data shown in Table 4 were obtained by methods in which a temperature above 650°C. was used. No comparisons can be made, since the number of representative analyses is too small and there is too much overlapping of values.

The data for sample 1 indicate that the better methods would use, in addition to temperatures above 650°C., a gas sample burner with two burnings, a gasometer, and 1.1% gas volume correction, and would take the temperature of the air as that of the gas in the nitrometer. The data for sample 2 indicate that the methods which use the alternates of these

TABLE 4.—*Nitrogen data obtained for samples 1 and 2 by the Dumas method*

	NICOTINIC ACID (1)			BENZYL-ISO-THIOUREA HYDROCHLORIDE (2)		
	NUMBER OF SAMPLES	\bar{X}	S	NUMBER OF SAMPLES	\bar{X}	S
		<i>per cent</i>			<i>per cent</i>	
Gas burner	18	11.41	0.055	16	13.97	0.152
Electrical burner	20	11.32	0.134	23	13.90	0.190
Sample burned twice	18	11.41	0.055	16	13.97	0.152
Sample burned once	20	11.32	0.134	23	13.90	0.190
Gasometer	17	11.41	0.063	17	13.97	0.145
No gasometer	21	11.33	0.134	22	13.90	0.197
1.1% gas volume correction	15	11.41	0.071	15	13.97	0.152
2% gas volume correction	12	11.29	0.158	12	13.78	0.155
Temperature measured in air	12	11.43	0.067	21	14.03	0.145
Temperature measured in liquid	26	11.33	0.121	18	13.81	0.145
Total samples	38	11.36	0.118	39	13.93	0.179
Theoretical values		11.38			13.82	

operations would be the better. This reversal of suitability of methods is improbable, and can no doubt be accounted for. Of these operations, the only arbitrary means of increasing or decreasing the per cent nitrogen is the correction applied to the gas volume. The mean nitrogen value for sample 2 after a 1.1% volume correction had been made, was too high by 0.15%. It seems unlikely that any of the other variations listed would cause this high value. Therefore, a 2% correction, which would give a mean value only 0.04% low, is apparently the more nearly correct and perhaps should be used in all cases. The reason that the 1.1% correction gave values nearer the theoretical value for nicotinic acid may be that this smaller correction compensated for some nitrogen not recovered from this refractory material.

Summary for Dumas nitrogen.—The study has shown that for satisfactory results by the Dumas method, the minimum temperature is 650°C.

A 2% volume correction appears to be required instead of the often-used 1.1% correction, but further work is necessary to prove this point and to establish preferences for other variations in the procedure.

The values submitted by the various collaborators for carbon, hydrogen, and nitrogen are presented graphically in Figures 15 to 19. No attempt has been made to analyze the values obtained from each laboratory separately.

The collaborators in these studies are listed below.

Collaborators on nitrogen analysis:

Alicino, J. F., Squibb Institute for Medical Research
 Jones, G. A., E. I. du Pont de Nemours and Company
 Brunner, A. H., Ansco
 Ketchum, D. E., Eastman Kodak Company
 Powers, D. A., Celanese Corporation of America
 Ogg, C. L., Eastern Regional Research Laboratory
 Hegeman, B., The Texas Company
 Dutton, C. D., Picatinny Arsenal
 Grodsky, J., Ortho Research Foundation
 Milner, R. T., Northern Regional Research Laboratory
 Blackman, S. W., The Wellcome Research Laboratories
 Sundberg, O. E., Calco Chemical Division, American Cyanamid Company
 Wagner, E. C., University of Pennsylvania

Collaborators on carbon and hydrogen analysis:

Kuck, J. A., American Cyanamid Company
 Owens, J. K., E. I. du Pont de Nemours and Company
 Sievers, D. C., Tennessee Eastman Corporation
 Rachele, J. R., Cornell University Medical College
 Paulson, R. A., National Bureau of Standards
 Brown, L. E., Southern Regional Research Laboratory
 Huffman, E. W. D., Huffman Microanalytical Laboratories
 Conard, V. A., Oakwold Laboratories
 Feldman, J. R., General Foods Corporation
 Hallett, L. T., General Aniline and Film Corporation
 Hynes, W. A., Fordham University
 Clark, H. S., Illinois State Geological Survey
 Aluise, V. A., Hercules Powder Company
 Butler, A. Q., Mallinckrodt Chemical Works
 Shreve, L. S., Smith, Kline and French Laboratories
 Streeter, K. B., Sharp and Dohme, Inc.
 Steyermark, Al, Hoffman-La Roche, Inc.
 Means, J. A., Charles Pfizer and Company, Inc.

RECOMMENDATIONS*

It is recommended that studies be continued on methods for the micro determination of carbon and hydrogen, and for nitrogen, by the Kjeldahl and Dumas procedures. Proposals based on the results of this year's work will be made concerning the procedures to be studied in the next year.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 56 (1949).

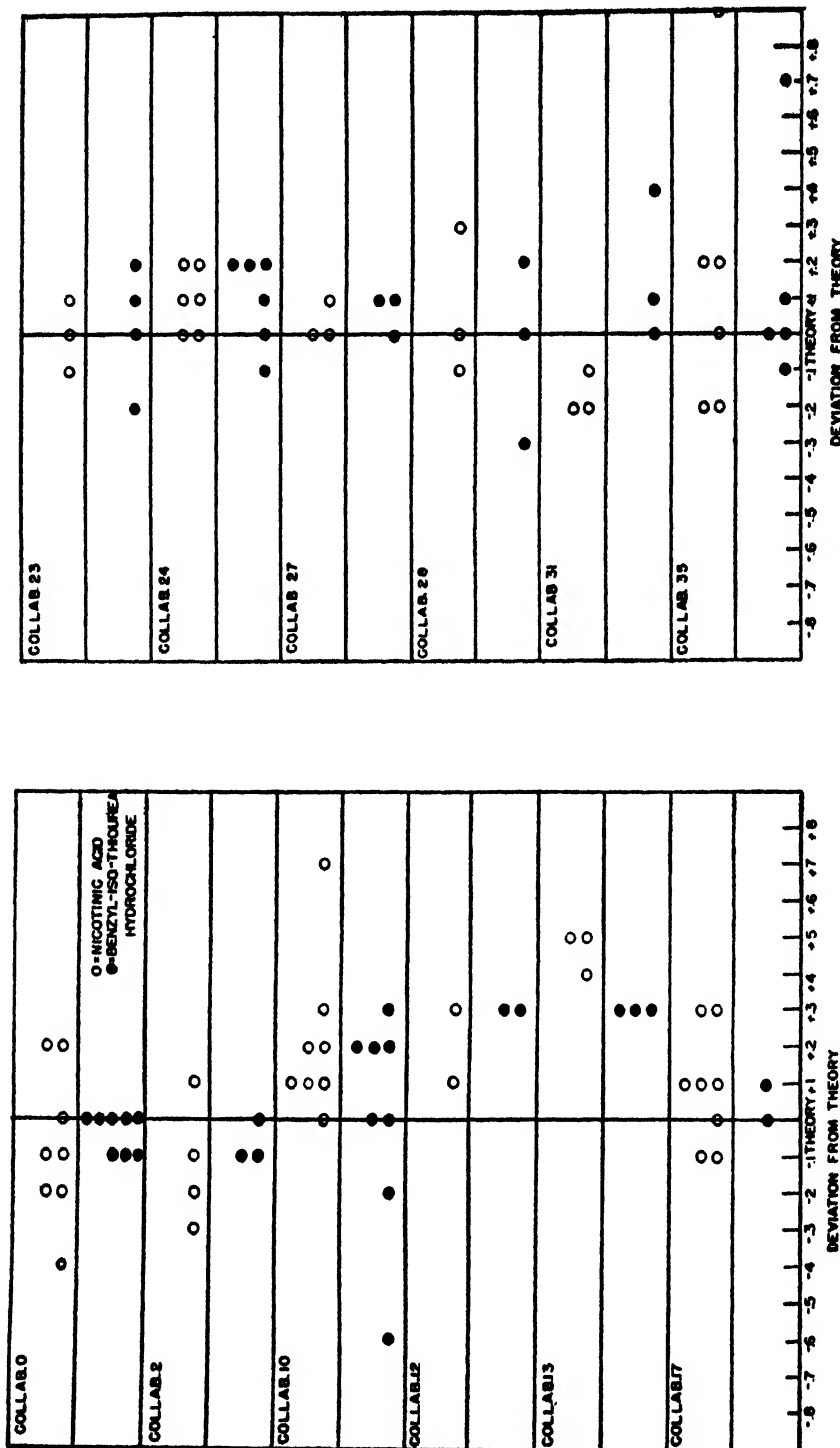


Fig. 15.—Continued.

Fig. 15.—Distribution of carbon values from the different collaborators.

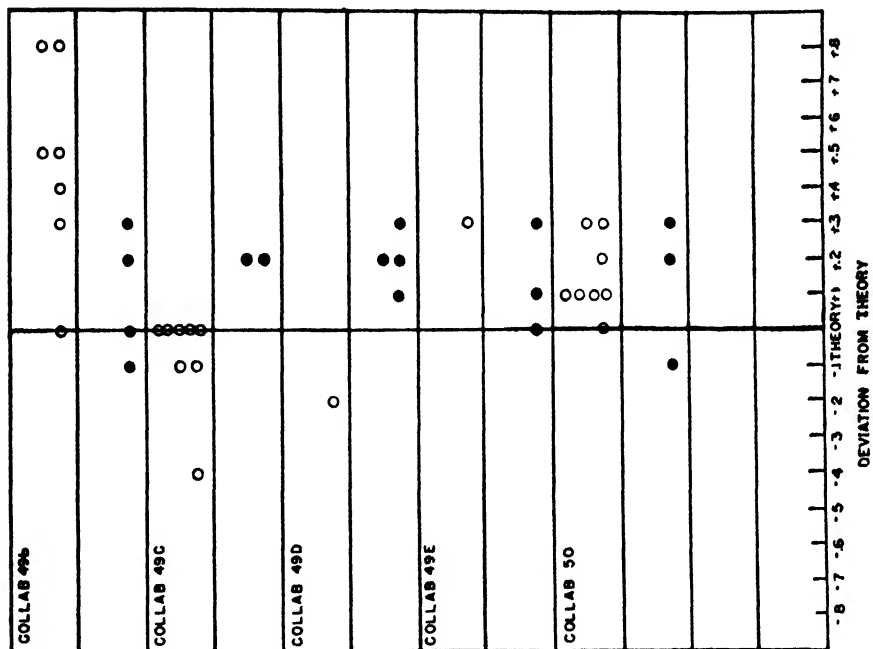


Fig. 15.—Continued.

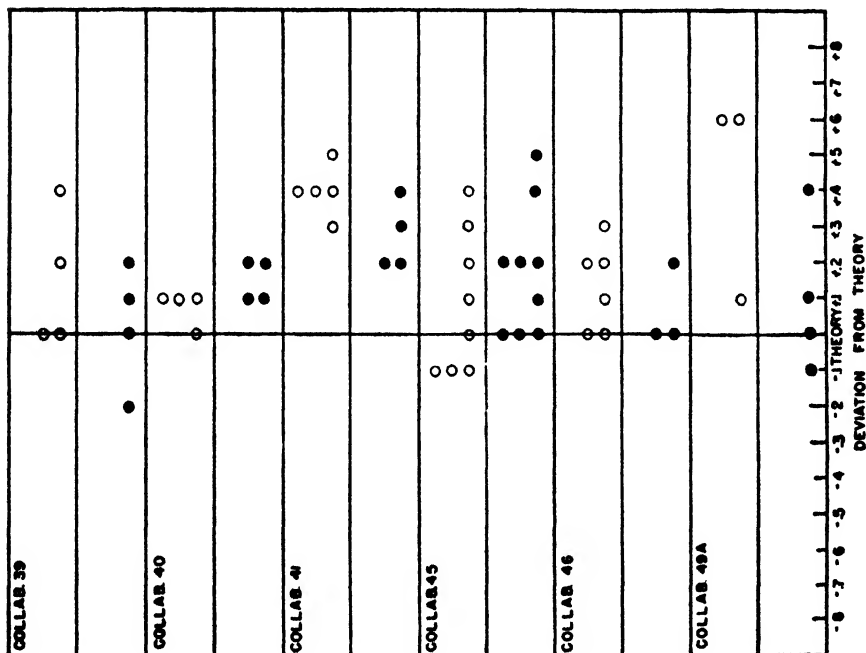


Fig. 15.—Continued.

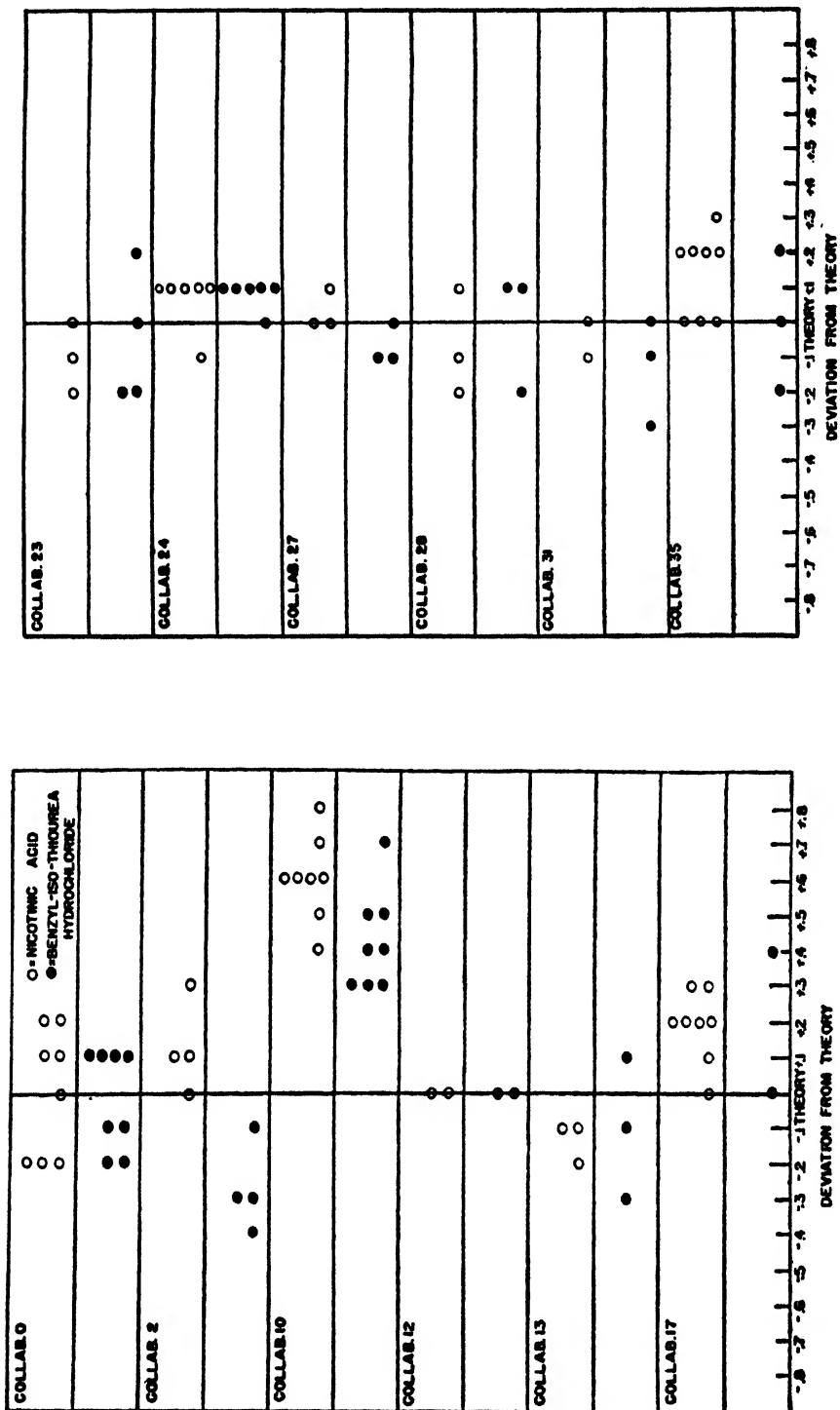


Fig. 16.—Distribution of hydrogen values from the different collaborators.

Fig. 16.—Continued.

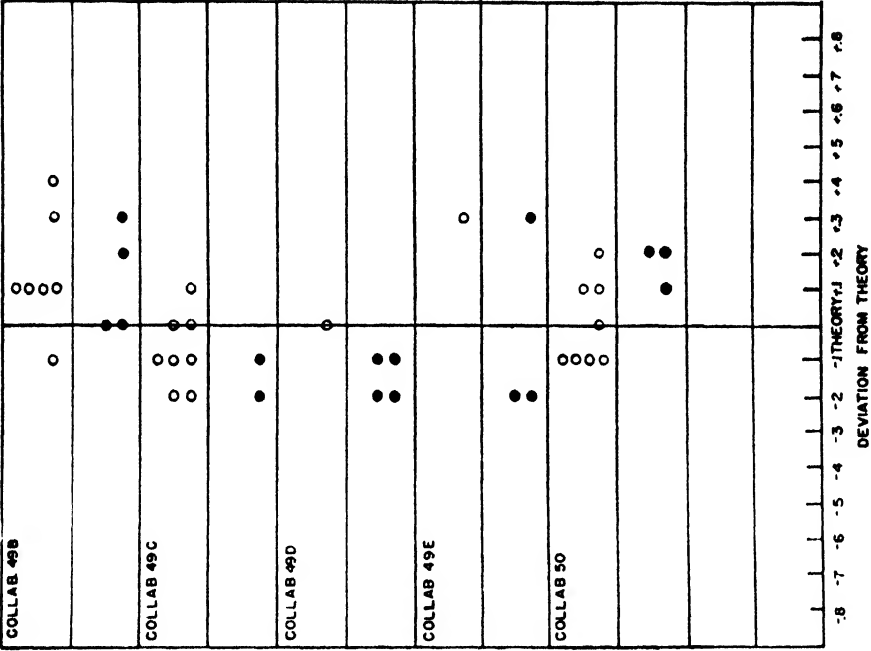


Fig. 16.—Continued.

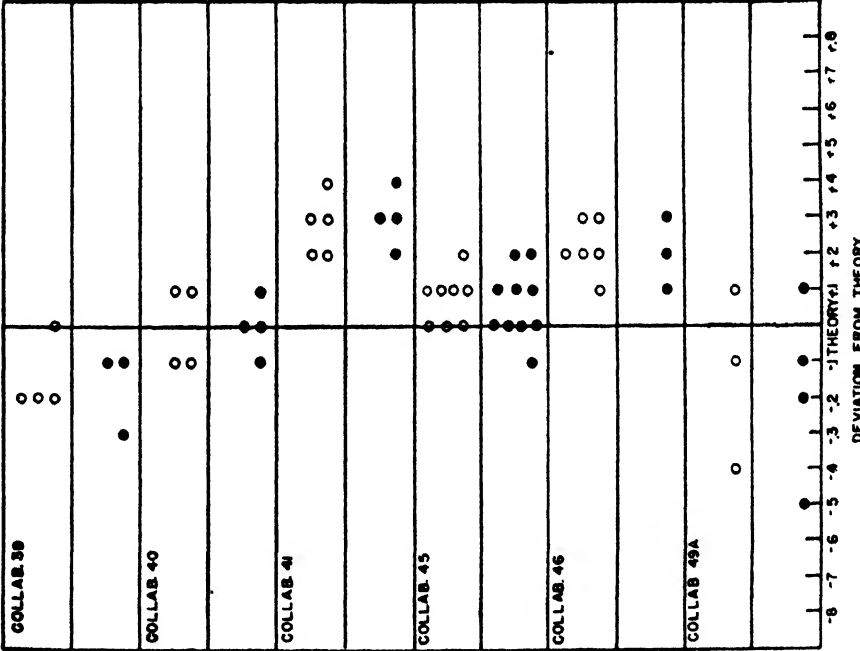


Fig. 16.—Continued.

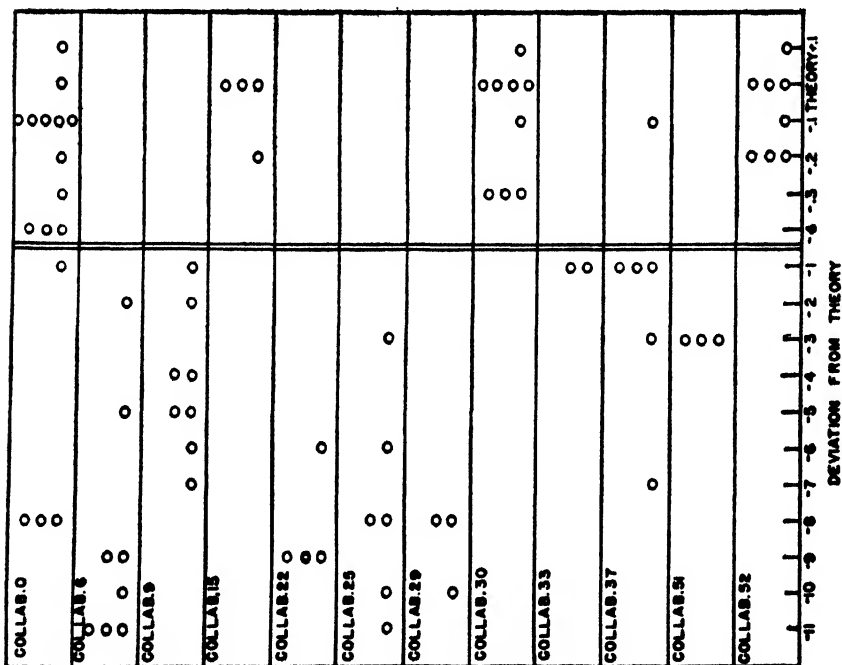


Fig. 17.—Distribution of Kjeldahl nitrogen values for nicotinic acid from the different collaborators.

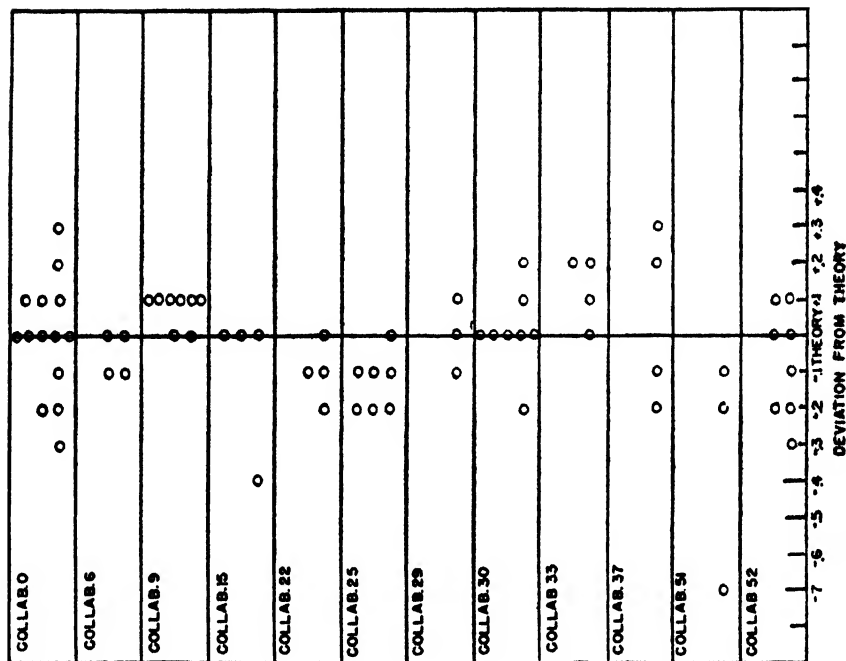


Fig. 18.—Distribution of Kjeldahl nitrogen values for benzyliso-thiourea hydrochloride from the different collaborators.

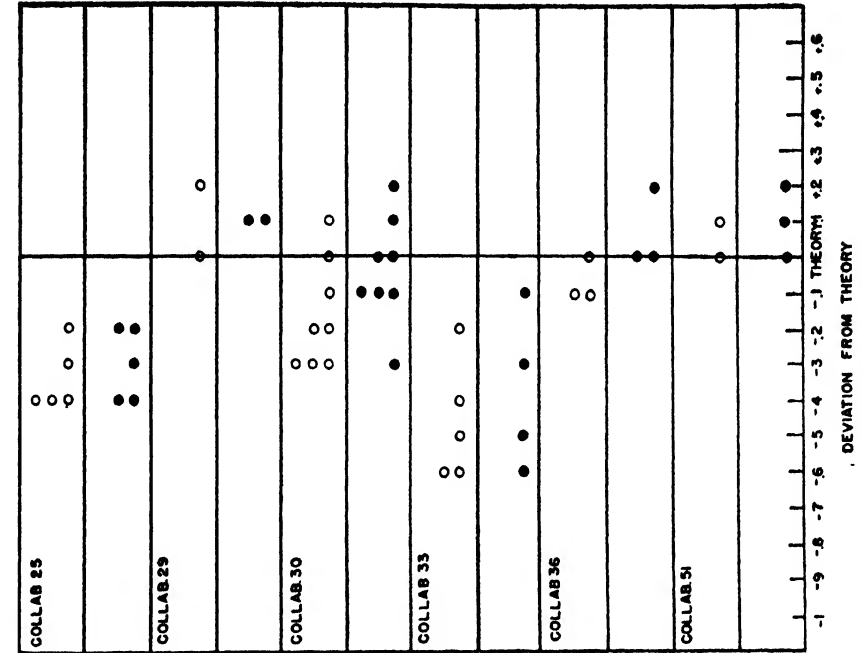


FIG. 19.—Continued

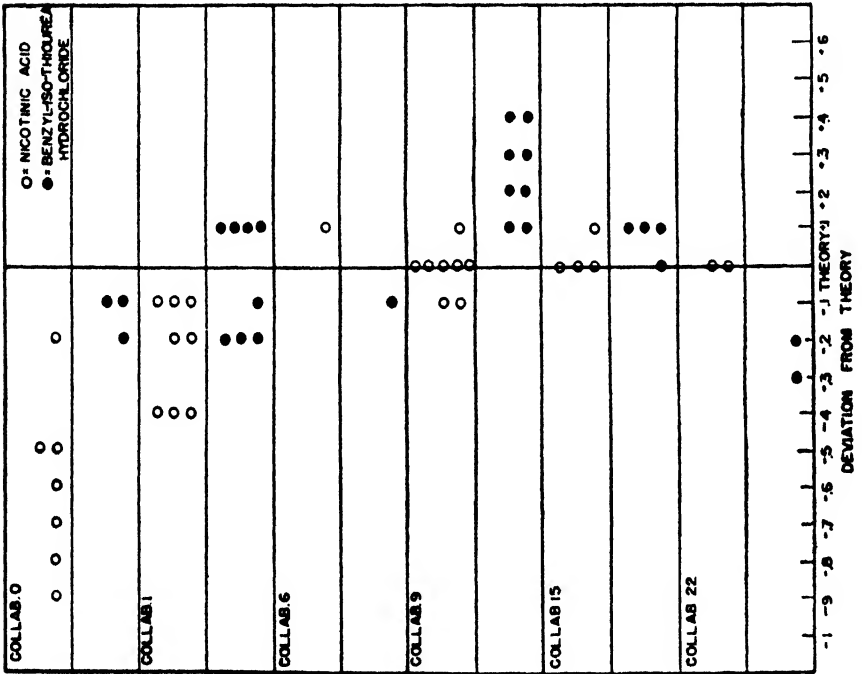
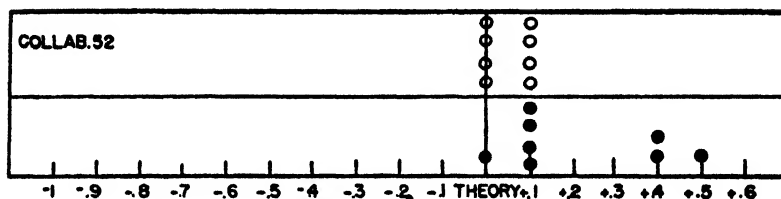


Fig. 19.—Distribution of Dumas nitrogen values from the different collaborators.



DEVIATION FROM THEORY

Fig. 19.—Continued.

The paper entitled "Kjeldahl Determination of Nitrogen in Refractory Materials," by C. O. Willits, M. R. Coe, and C. L. Ogg, is published in *This Journal*, 32, 118 (1949).

REPORT ON STANDARD SOLUTIONS

By H. G. UNDERWOOD (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Referee*

RECOMMENDATIONS*

Titanium Trichloride Solutions.—A report was received covering collaborative study of the three procedures of standardization reported on last year. The Associate Referee recommends that Method II, titanium trichloride solutions, 21.37, substituting potassium dichromate for potassium permanganate, be made official, first action. The Referee concurs.

Potassium Dichromate Solutions.—The Associate Referee compared the oxidometric strength of five commercial potassium dichromates with the Bureau of Standards product. He concludes that the findings may justify the preparation of standard solutions from assayed stocks of commercial potassium dichromate rather than from laboratory recrystallized potassium dichromate. The Referee suggests that the Associate Referee consider at least one recrystallization to insure uniformity. It is recommended that the subject be continued.

Standard Buffer Solutions.—No report was received. It is recommended that the subject be continued.

Hydrochloric Acid Solutions.—The method for the standardization of hydrochloric acid with standard sodium hydroxide was adopted as official, first action, at the 1938 meeting of the Association. It is recommended that the method be adopted as official, final action.

Sulfuric Acid Solutions.—The method for the standardization of sulfuric acid by the standard borax method was adopted as tentative at the 1939 meeting of the Association. Although the method was studied

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 46 (1949).

collaboratively in 1940, and the Associate Referee recommended that the method be adopted as official, first action, the Referee failed to discuss this recommendation in his report. It is recommended that the method be adopted as official, first action.

REPORT ON STANDARD POTASSIUM DICHROMATE SOLUTIONS

By GEORGE MCCLELLAN (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

The Sixth Edition of *Methods of Analysis*, A.O.A.C., (43.29, p. 810) specifies that tenth-normal solution of sodium thiosulfate be standardized against potassium dichromate that has been thrice recrystallized and dried at 200°C. Since publication of the Sixth Edition, the Bureau of Standards has made available a standard preparation of potassium dichromate.

A study was undertaken to determine how many recrystallizations of analytical grade commercial potassium dichromate would be necessary to bring it to a strength equal to that of the Bureau of Standards product. Portions of five different brands of analytical grade potassium dichromate were secured from various government laboratories in the New Orleans Custom House. Each portion was dried for one hour in a weighing bottle at 100 degrees C. The Bureau of Standards potassium dichromate to be used for comparison was dried at 200°C. for two hours. A solution of approximately 0.1 *N* sodium thiosulfate was made up according to sec. 43.28 of *Methods of Analysis*. Its normality was then ascertained to five places by titration against the freshly dried Bureau of Standards potassium dichromate. Naturally, it is impossible to determine normality to five places using an ordinary 50-ml straight tube buret and titrating a 0.20 to 0.23 g portion of potassium dichromate as directed in 43.29. A far more sensitive standardization was devised as follows:

PROCEDURE

Weigh into a glass-stoppered 500-ml Erlenmeyer flask enough $K_2Cr_2O_7$ to have a titre of between 100.5 and 102.0 ml of the standard thiosulfate (0.4929–0.5002 g for a 0.1 *N* soln). Completely dissolve in 100 ml of H_2O . Add 4.0 g KI and swirl until dissolved. Add 4.0 ml of HCl, stopper flask, mix by swirling, and allow to set in the dark for 10 min. Cool flask for about a min. in ice-water. While constantly swirling flask, add with a 100-ml pipet exactly 100 ml of the standard thiosulfate. Add 5 ml of 0.5% starch soln, and complete the titration with more of the standard thiosulfate added from a 10-ml micro-buret. End point is from a bluish green to a clear green. The change takes place within 0.01 ml. Record titre to the nearest hundredth of a ml.

EXPERIMENTAL

Three standardizations of the thiosulfate against the Bureau of Standards potassium dichromate gave normalities of 0.10439, 0.10438, and

0.10438, respectively. A value of 0.10438 was taken as the true normality of the solution, and the Bureau of Standards product was taken to be 100.00% pure potassium dichromate. This same thiosulfate solution was then standardized in the same manner against each of the five commercial brands of dichromate being tested. Purity of each commercial dichromate was calculated as follows:

Normality of $\text{Na}_2\text{S}_2\text{O}_3$ by B. of S. $\text{K}_2\text{Cr}_2\text{O}_7 \times 100$
 Normality of $\text{Na}_2\text{S}_2\text{O}_3$ by $\text{K}_2\text{Cr}_2\text{O}_7$ being tested equals % purity of the $\text{K}_2\text{Cr}_2\text{O}_7$ being tested.

Results were as shown below:

BRAND ¹ $\text{K}_2\text{Cr}_2\text{O}_7$	DETN. NO.	GRAMS $\text{K}_2\text{Cr}_2\text{O}_7$ TITRATED	TITRE IN ML. STD. $\text{Na}_2\text{S}_2\text{O}_3$	APPARENT NORMALITY OF $\text{Na}_2\text{S}_2\text{O}_3$	PURITY OF $\text{K}_2\text{Cr}_2\text{O}_7$ AS COMPARED WITH B. OF S. PRODUCT
					per cent
B of S	1	0.5212	101.81	0.10439	—
	2	0.5182	101.23	0.10438	—
	3	0.5165	100.90	0.10438	—
A	1	0.5175	101.10	0.10438	100.00
	2	0.5172	101.04	0.10438	100.00
B	1	0.5165	100.84	0.10444	99.94
	2	0.5174	100.98	0.10448	99.90
C	1	0.5173	101.10	0.10434	100.04
	2	0.5164	100.88	0.10438	100.00
	3	0.5171	101.01	0.10439	99.99
D	1	0.5172	101.08	0.10434	100.04
	2	0.5170	101.01	0.10437	100.01
E	1	0.5170	100.98	0.10440	99.98
	2	0.5170	100.98	0.10440	99.98

¹ Brands A, B, C, D, and E are Baker, Conray, Elk, Gennert, and Mallinckrodt, although not in that order.

CONCLUSIONS

It appears that four out of five of the commercial dichromates tested are already so pure that any improvement by recrystallization could probably not be determined by volumetric assay. Even the comparatively impure "Brand B" has an effective strength of 99.90 per cent or better.

The procedure outlined above can be used to determine the oxidimetric strength of any given batch of potassium dichromate as compared with that of Bureau of Standards potassium dichromate. From the data given, it appears that the analytical error will not exceed one part in two thousand.

RECOMMENDATIONS*

It is recommended that the procedure given above be utilized in a collaborative study to determine the oxidimetric strengths of two or more commercial "analytical grade" potassium dichromates in terms of the Bureau of Standards product. If collaborators agree within 0.05 per cent, then it will later be recommended that a procedure for assaying stocks of laboratory potassium dichromate, rather than a routine procedure of recrystallization, be incorporated in the *Methods of Analysis*.

REPORT ON STANDARDIZATION OF
TITANIUM TRICHLORIDE

By JUANITA E. BREIT (Food and Drug Administration, Cincinnati, Ohio),
Associate Referee

Methods for the preparation of solutions and for standardizing titanium trichloride have been previously described.¹ Briefly, Method I is the A.O.A.C. method using potassium permanganate, ferrous ammonium sulfate, and ammonium thiocyanate; Method II is the same, but substituting potassium dichromate for potassium permanganate; Method III uses potassium dichromate and diphenylamine indicator.

In view of the unstable nature of titanium trichloride, it was considered inadvisable to send out solutions of the chemical for standardization; instead, at each station doing collaborative work, one chemist prepared the titanium solution which was then standardized by all collaborators, each using the three methods as outlined, and each preparing his own potassium permanganate and potassium dichromate solutions.

LIST OF COLLABORATORS

Station 1—Food and Drug Administration, Color Certification Section, Washington, D. C.

Alice B. Caemmerer; Meyer Dolinsky; S. S. Forrest; Nathan Gordon; Charles Graichen; L. S. Harrow.

Station 2—Food and Drug Administration, Chicago, Ill.

Daniel Banes; Harold F. O'Keefe; Robert Stanley.

COMMENTS OF COLLABORATORS

1. Method III gave decidedly higher results than the others. The results are also more erratic. Methods I and II give better checks.

2. Method III gives a higher titre than the other two and the end point requires some patience to ascertain accurately; otherwise it is no more erratic than potassium permanganate.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 46 (1949).

¹ *This Journal*, 31, 573 (1948).

TABLE 1.—*Results of Collaborative Work—Stations 1 and 2*

COLLABORATOR	METHOD I		METHOD II		METHOD III	
	NORMALITY OF TiCl ₃	AVE.	NORMALITY OF TiCl ₃	AVE.	NORMALITY OF TiCl ₃	AVE.
Station 1						
1	0.0941		0.0943		0.0948	
1	0.0941		0.0941		0.0950	
1	0.0940	0.0941	0.0939	0.0941	0.0955	0.0951
2	0.0946		0.0946		0.0955	
2	0.0950		0.0946		0.0955	
2	0.0943	0.0946	0.0941	0.0944	0.0952	0.0954
3	0.0941		0.0942		0.0949	
3	0.0941		0.0942		0.0950	
3	0.0939	0.0940	0.0942	0.0942	0.0949	0.0949
4	0.0937		0.0942		0.0948	
4	0.0938		0.0947		0.0948	
4	0.0942	0.0939	0.0943	0.0944	0.0947	0.0948
5	0.0944		0.0949		0.0952	
5	0.0942		0.0943		0.0948	
5	0.0941	0.0942	0.0945	0.0946	0.0948	0.0949
6	0.0937		0.0940		0.0957	
6	0.0940		0.0939		0.0957	
6	0.0938	0.0938	0.0937	0.0939	0.0963	0.0959
Average	0.0941		0.0943		0.0952	
Range	0.0937–0.0950		0.0937–0.0949		0.0947–0.0963	
Standard Deviation	0.00026		0.00023		0.00038	
Station 2						
7	0.1233		0.1237		0.1238	
7	0.1232		0.1237		0.1240	
7	0.1232	0.1232	0.1237	0.1237	0.1239	0.1239
8	0.1240		0.1238		0.1239	
8	0.1238		0.1236		0.1239	
8	0.1239	0.1239	0.1238	0.1237	0.1240	0.1239
9	0.1238		0.1237		0.1238	
9	0.1240		0.1237		0.1239	
9	0.1241	0.1240	0.1237	0.1237	0.1238	0.1238
Average	0.1237		0.1237		0.1239	
Range	0.1232–0.1241		0.1236–0.1238		0.1238–0.1240	
Standard Deviation	0.00035		0		0	

3. If the sulfuric acid and water have any blanks due to dissolved oxygen it cannot be determined in Method III, as the indicator will give no color unless chro-

mate is added. Any correction for a blank in Method III would result in a higher normality and a wider deviation from the first two methods.

4. The end point in Method III is difficult to determine since the purple color returns. Method II appears to be preferable.

5. Method III appears to give somewhat higher values than the two standard methods. It has the advantage of not requiring a blank determination, otherwise it is essentially the same as Method II.

6. Method III affords some difficulty. The diphenylamine indicator doesn't give a permanent end point; it has a tendency to fade out. Somewhat higher results are indicated using the diphenylamine indicator.

7. Prefer potassium dichromate titration with ferrous ammonium sulfate indicator because of ease of handling and relative stability of the potassium dichromate. Diphenylamine indicator seems to shift the stoichiometric point and gives a higher titanium trichloride factor. Diphenylamine titration must be run slowly to eliminate possibility of indicator precipitation.

8. Prefer Methods II and III to I.

It is recommended*—

That Method II be adopted as official, first action.

No report was given on buffer solutions.

REPORT ON COSMETICS

By G. ROBERT CLARK (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

RECOMMENDATIONS†

The Referee recommends—

(1) That the following topics on which no reports were received be continued:

Cosmetic Creams

Deodorants and Anti-perspirants

Depilatories

Hair Dyes and Rinses

Moisture in Cosmetics

(2) That the following topics be discontinued:

Alkalies in Cuticle Removers

Mercury Salts in Cosmetics.—It is believed that the methods described or reported as being studied under the topic "Miscellaneous Drugs" can be applied without modification to cosmetics.

Hair Straighteners

The Referee concurs in the following recommendations of Associate Referees.

(1) That the topic "Mascaras, Eyebrow Pencils, and Eye Shadows" be continued.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 46 (1949).

† For report of Subcommittee B and action of the Association, see *This Journal*, 32, 50 (1949).

- (2) That the topic "Cosmetic Skin Lotions" be continued.
 - (3) That the proposed methods for "Pyrogallol in Hair Dyes" be adopted as official, first action, and the topic closed.
 - (4) That the proposed methods for the "Analysis of Face Powder" be adopted as official, first action, with the following changes:
 - (a) That the methods be adopted individually, as methods for the various constituents of face powder rather than as an entire method for the analysis of face powder. This will provide for the addition of methods for other constituents, not included in the study to date, should the necessity for them appear.
 - (b) The proposed method for stearate be designated "Fats and Fatty Acids as Stearic Acid."
- The Referee further recommends reassignment of this topic, since the present Associate Referee has so requested.
- (5) That the topic "Sun Tan Preparations" be made the subject of study by an Associate Referee.

REPORT ON PYROGALLOL IN HAIR DYES

By CURTIS R. JOINER (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

A colorimetric method for the determination of pyrogallol in liquid hair dyes has been adopted as tentative (1, 2). In a later report (3) the writer discussed some of the problems involved in the extraction of pyrogallol from henna mixtures and recommended that further work be done on the method.

The method as previously outlined involved (1) extracting the pyrogallol from henna mixtures with ethyl acetate and evaporating the extract to dryness; (2) taking up the residue with water, adding alumina cream and filtering; and, (3) determining the pyrogallol in the filtrate by the tentative colorimetric method. The main objection to this method was the high and variable blanks obtained with it from henna powder. Several variations of this method were discussed.

Some recent work has shown that the use of alumina cream in clarifying the aqueous solution of the extracted material affected the slope of the curve. Extracting the pyrogallol from the clarified filtrate with ether prior to its colorimetric determination served to reduce the blank materially and to eliminate the effect of alumina cream on the development of color.

In order to integrate this method with the tentative one for liquid dyes, it was desirable, and in some cases necessary, to rewrite portions of the latter. The method as finally submitted to collaborative study is given below:

PYROGALLOL IN HAIR DYES

Qualitative Test

Add 5–10 ml of sample to separatory funnel containing ca 0.5 g of NaHSO_3 and extract with 2 or 3 successive 30-ml volumes of ether. Filter ether extracts thru cotton and evaporate to dryness on steam bath. Dry in oven at 100°C for 30–60 min. Pulverize residue, mix well, and take melting point. If it does not melt between 131° and 134°C , sublime and again take melting point, which should fall within above range. Mix small portion of residue with equal quantity of sublimed pyrogallol and determine the melting point; it should not change.

Quantitative Determination

REAGENTS

Ferrous Tartrate Reagent.—Dissolve 1.00 g of sodium potassium tartrate (Rochelle salt) and 0.200 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in water and dilute to 100 ml in a volumetric flask. PREPARE FRESH DAILY.

Sodium Acetate Solution.—Dissolve 15.00 g $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ in water, bring to room temp. and dilute to 100 ml.

Alumina Cream.—34.19(b).

Standardization.—To six 100 ml volumetric flasks add from buret 2.50, 5.00, 7.50, 10.00, 12.50, and 15.00 ml of standard pyrogallol solution (Reagent Grade, 0.2000 g/500 ml). Develop color as follows on not more than three standards at a time, and make readings within 15 min. after color is developed. Pipet into flasks 10 ml each of Na acetate soln and ferrous tartrate reagent, dilute to volume and mix. Using 1 cm or $\frac{1}{2}$ inch cells, measure optical density of solutions with photometer or spectrophotometer at 540 m μ . With neutral wedge filter photometer, filter designated No. 56 (5.8 mm of Corning didymium #512, 2.0 mm of Jena VG 3, 2.0 mm of Jena BG 18, and 4.5 mm of Corning yellow shade yellow #351) is more suitable than filter No. 54. With filter photometers obtain zero point by reading "blank" soln containing 10 ml of Na acetate and ferrous tartrate reagents in 100 ml. Draw standard curve, plotting concentration of pyrogallol against photometer readings, on large scale graph paper so that pyrogallol can be read to 0.01 mg. A straight line should be obtained between concentrations of 1 mg and 6 mg per 100 ml. With spectrophotometer use freshly prepared "blank" as reference soln. Draw standard curve as directed above, or, if straight line passing thru origin is obtained, the average value of k may be calculated from the formula

$$k = \frac{D}{c}$$

where

k is extinction coefficient

D is measured optical density of solution

c is concentration of pyrogallol in mg per 100 ml

This value of k may be used to calculate the concentration of unknowns directly from optical density readings.

Liquid Dyes

Extract a convenient aliquot of sample (usually 10 ml are sufficient) by one of the following methods. In handling sample give it a minimum of exposure to air, as pyrogallol is readily oxidized.

1. *Continuous Extraction.*—Pipet sample aliquot into suitable continuous extractor containing ca 0.3 g of NaHSO_3 . Extract with ether until pyrogallol is completely removed (3–7 hours, depending upon efficiency of extractor). Determine time

required for each extractor under a certain set of conditions by extracting an aqueous soln of known pyrogallol content or by testing for complete extraction as follows: After the extraction is thought to be complete, remove flask containing ether and replace it with one containing fresh volume of ether and continue extraction for 30–60 min. Treat this extract as directed below and, use 50 ml aliquot of filtrate to develop color. Evaporate ether extract on steam bath to volume of 8–10 ml and continue evaporation at temp. not exceeding 40° until odor of ether is completely gone. Dissolve residue in 20 ml of water and wash completely into 100 ml volumetric flask. Dilute to volume and mix. (If the liquid sample contained chlorophyll treat residue from ether extraction as described for residue obtained from evaporation of ethyl acetate extract in method for henna powder dyes, beginning, "Add ca 10 ml water to beaker and loosen the residue. . ."). Filter thru dry paper and discard first 20 ml of filtrate. (If determination cannot be completed same day extraction is made, let ether extract stand overnight, preferably in refrigerator, before ether is evaporated. Do not let aqueous soln stand overnight.) Use suitable aliquots of filtrate to develop color as directed under "Standardization," beginning "Pipet into flasks. . . ." If 5 ml aliquot contains more than 6 mg of pyrogallol, make suitable dilution in volumetric flask and use aliquots of diluted soln to develop color. For final calculation use average of results obtained on at least two aliquots of different sizes, preferably containing between 2 mg and 5.5 mg of pyrogallol. Calculate to g/100 ml in original sample.

2. *Extraction in Separatory Funnels.*—Pipet sample into 125 ml separatory funnel containing ca 0.3 g of NaHSO_4 and extract 6 times with ether. For each extraction use volume of ether equal to 3 or 4 times volume of sample and shake vigorously for one min. Filter ether extracts successively thru cotton wet with ether. (Six extractions carefully made will completely remove the pyrogallol. If desired, a 7th may be made and used to test for complete extraction as described under "Continuous Extraction.") Evaporate the combined ether extracts as directed under "Continuous Extraction."

Henna Powder Mixture

Weigh 0.9 to 1.1 g of thoroughly mixed sample in paper extraction thimble. Cover sample with small piece of cotton and place thimble in Soxhlet extractor. If temperature and humidity conditions are such that water will condense on condenser, connect tube containing drying agent to outlet of condenser. Extract 5 hours with ethyl acetate having minimum purity of 99% (N.F. VIII or better quality). Boil at such rate that solvent siphons off 15 to 20 times per hour. If ethyl acetate extract is clear, evaporate to dryness as directed below. If extract contains any sediment, evaporate to ca 75 ml if necessary, cool to room temperature, and completely transfer to 110 ml glass-stoppered volumetric flask. Dilute to volume and mix. Filter thru dry paper, taking precautions to prevent evaporation of solvent. Pipet 100 ml of filtrate into 250 ml beaker and evaporate to volume of ca 5 ml on hot plate or steam bath. Continue evaporation to complete dryness at temperature not exceeding 40°. Add 10 ml water and loosen residue with stirring rod. Pour into 50 ml volumetric flask. Rinse beaker 4 or 5 times with small volumes of water and add rinsings to flask. Add 1.2 ml alumina cream, dilute to volume, mix and filter thru dry paper. Extract 25 ml by one of methods given under "Liquid Dyes." (If extraction cannot be started immediately add ca 0.4 g of NaHSO_4 to filtrate and hold no longer than overnight.) Calculate to per cent pyrogallol in original sample.

EXPERIMENTAL

Recovery Experiments.—Approximately one gram samples of henna were weighed in paper extraction thimbles, and accurately weighed

quantities of pyrogallol ranging from about 40 to 150 milligrams were added to the thimbles. Three different lots of henna were used. By preparing the samples in this manner, the problem involving oxidation of pyrogallol when mixed with henna powder was entirely avoided. The results are given in Table 1. On thirteen determinations an average recovery of 99.3 per cent was obtained, with a range from 97.9 to 100.2 per cent.

TABLE 1.—*Recovery experiments*

PYROGALLOL ADDED		PYROGALLOL RECOVERED	
milligrams		milligrams	per cent
42.0		41.3	98.3
47.8		47.1	98.5
56.0		55.7	99.5
53.1		53.2	100.2
60.7		60.4	99.5
50.2		49.9	99.4
65.0		65.1	100.2
106.0		104.4	98.5
149.3		148.7	99.6
52.7		51.6	97.9
72.0		71.7	99.6
63.4*		63.5	100.2
123.8*		122.9	99.3
Average			99.3

* No henna powder was added to these two samples.

Blanks.—Eight blank determinations were run on the three lots of henna used in the above recovery experiments. These results ranged from 0.073 to 0.118 per cent, with an average of 0.107 per cent, calculated as pyrogallol. Theoretically, with a blank of this magnitude, the recoveries listed in Table 1 should range from 0.7 to 2.5 per cent high. Since there was no tendency to get high results, either there must be a compensating loss of pyrogallol, or the presence of pyrogallol alters the blank values of the henna powder.

Storage Experiments.—Pyrogallol recoveries obtained on samples after 12 to 38 months' storage are given in Table 2. Filter cel was used for two of the samples in order to compare the stability of pyrogallol when mixed with an inert material with its stability in henna mixtures. Lawsone, 2-hydroxy-1, 4-naphthoquinone, the active constituent of henna for hair-dyeing purposes (4), was added to two of the samples to see what effect, if any, it would have on the rate of oxidation of pyrogallol. In both cases the filter cel samples (II and IV) showed about five times as much loss of pyrogallol as the corresponding henna samples (I and III). The loss of pyrogallol in the two samples containing lawsone (I and II) was considerably less than that in the corresponding samples with no added law-

sone (III and IV). Continuing the ethyl acetate extraction of the samples for two to three hours beyond the five-hour period designated in the method resulted in the recovery of no more pyrogallol.

TABLE 2.—*Storage experiments*

SAMPLE NO.	COMPOSITION OF SAMPLES	APPROX. AGE WHEN ANALYZED	PYROGALLOL FOUND	RECOVERY
		<i>months</i>	<i>per cent</i>	<i>per cent</i>
I	Egyptian Henna* Lawson 1.0% Pyrogallol 5.51%	13	4.93	89.5
II	Filter cel Lawson 1.0% Pyrogallol 5.55%	13	2.25	40.5
III	Egyptian Henna Pyrogallol 5.54%	13	4.66	84.1
IV	Filter cel Pyrogallol 5.52%	13	1.57	28.4
V	Bronzing Henna* Pyrogallol 5.53%	12	4.91 4.85	88.8 87.7
VI	Egyptian Henna Copper Sulfate 5% Burnt Sienna 4% Pyrogallol 5.52%	12	4.82	87.3
5	Henna† Pyrogallol 5.24%	38	3.97	75.8

* These two lots of henna were purchased from a national distributor of beauty shop supplies. A representative of the firm stated that both were Egyptian henna, but that the bronzing henna had been treated so that it would color the hair a different shade. The method of treatment was not known.

† This lot was purchased from another distributor and was labeled, "Egyptian Henna."

An absorption curve obtained with a Beckman spectrophotometer was similar to that of Mattil and Filer (5) for the color produced by gallic acid and the ferrous tartrate reagent. The curve had a very broad absorption band with a maximum at 540 millimicrons. However, with a neutral wedge photometer (6), filter No. 56 was superior to No. 54. The No. 56 filter gave greater absorption values, and the field was brighter and was easier to match than with filter No. 54. The measurements reported here were made with the spectrophotometer using a spectral band width of about 1.3 millimicrons.

COLLABORATIVE WORK

Three samples for collaborative study were prepared as follows:

No. 1

Pyrogallol	9.36 per cent
Burnt sienna	4.5 per cent
Copper sulfate	5.2 per cent
Egyptian Henna	

No. 2

Pyrogallol	5.14 per cent
Bronzing Henna	

No. 3

Pyrogallol	20.500 grams
Sodium bisulfite	10 grams
Distilled water to make one liter.	

All ingredients for samples 1 and 2 were ground to pass a 60-mesh sieve. The samples were mixed in large jars on a revolving mixer for about ten hours. Each sample was divided into ten parts, and each part was placed in a small bottle. The writer analyzed portions from two bottles selected at random from both of the samples before they were sent out for collaborative study. The collaborators were asked to analyze the samples in

TABLE 3.—*Collaborators' results*

ANALYST	DATE ANALYZED	SAMPLE 1		SAMPLE 2		SAMPLE 3	
		PYROGALLOL FOUND	RECOVERY	PYROGALLOL FOUND	RECOVERY	PYROGALLOL FOUND	RECOVERY
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>g/100 ml</i>	<i>per cent</i>
C. R. Joiner	3/11/48	9.43	100.8	5.16	100.4	2.02	98.5
		9.36	100.0	5.10	101.0	2.02	98.5
1	4/ 1/48	9.30	99.4	5.08	98.8	2.00	97.6
		9.26	98.9	5.11	99.4	2.01	98.0
2	4/ 5/48	9.24	98.7	5.12	99.6	2.11	102.9
		9.26	98.9	5.09	99.0	2.11	102.9
						2.10	102.4
						2.11	102.9
3	4/13/48	9.13	97.5	4.89	95.1	2.09	102.0
		9.09	97.1	5.00	97.3	2.07	101.0
4	4/18/48	9.03	96.5	5.11	99.4	2.00	97.6
		9.04	96.6	5.00	97.3	2.01	98.0
5	5/11/48	8.94*	95.5	4.74*	92.2	2.00	97.6
		8.96*	95.7	4.86*	94.6	2.00	97.6
C. R. Joiner	5/19/48	8.63*	92.2	4.69*	91.2	—	—
C. R. Joiner	7/12/48	7.98*	85.3	4.47*	87.0	—	—
Averages		9.21	98.4	5.08	98.8	2.05	100.0
Standard Deviations		±0.13%		±0.084%		±0.046 g	
Range		0.40%		0.30%		0.11 g/100 ml	

* These results are not included in the averages. See explanation under "Discussion."

duplicate within a month if possible. The results are listed in Table 3. Samples 1 and 2 are similar in composition to commercial henna dyes. Samples similar to No. 3 have been subjected to collaborative study twice before, while this is the first such study for henna samples.

DISCUSSION

No significant comments were made by the collaborators. Apparently none of them had any difficulty in following the directions.

The results obtained by the collaborators are good when consideration is given to the nature of pyrogallol and the difficulties involved in isolating it from plant material. The recoveries on sample No. 1 show a steady decrease with increasing age of sample, and those on sample No. 2, with two exceptions, do the same. The samples were about five and one-half weeks old when analyst 4 made his determinations, and they were nine weeks old when analyst 5 made his. The determinations made by the Associate Referee one week later furnish ample justification for not including the results of analyst 5 in the averages used for evaluating the method. The apparent rate of loss of pyrogallol from these samples is considerably greater than that in any of the henna samples reported in Table 2. From these considerations it is probable that if all determinations had been made within two or three weeks, the results would have been better.

The discussion regarding loss of pyrogallol on aging does not apply to sample 3. The results obtained on this sample are approximately the same as those reported for similar samples in the past collaborative studies.

RECOMMENDATIONS*

It is recommended that the method presented in this report be adopted as official, first action, and that work on this subject be discontinued.

ACKNOWLEDGMENT

The writer wishes to express his appreciation to the following staff members of the field laboratories of the Food and Drug Administration who took part in this work as collaborators: S. H. Perlmutter and Gloria Getchell, Minneapolis, Minnesota; H. C. Van Dame, Cincinnati, Ohio; H. W. Conroy, Kansas City, Missouri; D. Banes, Chicago, Illinois; and F. M. Garfield, St. Louis, Missouri.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **32**, 50 (1949).

REPORT ON MASCARAS, EYEBROW PENCILS, AND EYE SHADOWS

By PAUL W. JEWEL (Max Factor & Co., 1666 North Highland Avenue, Hollywood, Calif.), *Associate Referee*

II. THE ANALYSIS OF MASCARA

A method for the analysis of mascara reported earlier¹ has been improved to such an extent as to make its submission for collaborative study desirable. Some changes in procedure have been made but the experimental material has not been changed, since this type of mascara is rather universally used in the cosmetic industry.

THE METHOD

Total Base

Weigh sample of mascara, ca 3.0000 g, wrap in filter paper (Munktell 00) turning ends in so as to make a seal, and tie with thread. Place this wrapped sample in a double thickness extraction thimble, plug opening with cotton, and extract with chloroform in a Soxhlet Extractor at such a rate that the siphon dumps about once every 10 min. Continue extraction for 8 hours. When the extraction has been completed, evaporate solvent on steam bath, dry in oven for 30 min., cool, and weigh. Repeat the drying in the oven until all odor of chloroform has been removed and weight remains constant. Report result as per cent total base. (When most of chloroform has been removed by evaporation, it will be found that the addition of a few ml of absolute alcohol will facilitate removal of the last traces.)

Triethanolamine

To total base contained in extraction flask, add 10 ml of 95% ethyl alcohol, and heat until base is dissolved or dispersed. Add 10.00 ml of 0.50 *N* sulfuric acid, heat to boiling, add 50 ml distilled water, heat until fats melt, and chill thoroly in the ice box. When mixture is thoroly chilled, filter thru paper into titration flask. Wash waxes several times with water and titrate filtrate with 0.50 *N* aqueous sodium hydroxide using methyl red as an indicator. Divide the net titration expressed as ml of *N* acid by 7.0 to get g of triethanolamine found. Express results in terms of per cent of triethanolamine in original sample.

Total Acid as Stearic Acid

Return filter paper from the determination of triethanolamine to the extraction flask, add 50 ml absolute alcohol, heat to dissolve and titrate with alcoholic potassium hydroxide 0.50 *N*, using phenolphthalein as indicator. Calculate total acid found as stearic acid, using 208 as the acid number for stearic acid, and report the result as per cent stearic acid.

In order to test the reliability of this method in the hands of other chemists, it was submitted to collaborative study. Samples were sent to six laboratories, five of which responded. Table 1 gives the results obtained by these collaborators.

¹"I. The Analysis of Mascara" was published in *This Journal*, 29, 32 (1946).

TABLE 1.—*Collaborative results*

COLLABORATOR	TOTAL BASE	TRIETHANOLAMINE	TOTAL ACID AS STEARIC ACID
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	73.50	11.41	24.60
2	72.20	11.70	24.30
3	72.64	10.65	18.15
4	73.55	12.50	26.25
5	73.53	11.12	24.80
Av.	73.08	11.48	23.62
Theory	75.09	11.99	31.04

COMMENTS OF COLLABORATORS

#3. "It would appear that eight hours is not sufficient for complete extraction of the mascara base."

This collaborator then proceeded to continue the extraction for 10 and 12 hour periods. The results of this additional extraction appear in Table 2, together with a 24 hr. extraction made by the Associate Referee.

TABLE 2.—*Additional extraction*

	TOTAL BASE	TRIETHANOLAMINE	TOTAL ACID AS STEARIC ACID
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
10 hours	73.54	11.22	24.68
12 "	74.05	11.79	26.54
24 "	73.94	11.26	26.50

The results for total base and for triethanolamine are not too precise from an analytical standpoint, but for mixtures of this sort they are fairly good. The results for total acid are too far from correct and indicate that some further treatment of the extracted base may be necessary. The time of extraction is important, and in order to make sure that this will be complete under any and all conditions, it is recommended that the extraction time be extended to 24 hours.

It is difficult to explain the lack of precision in results obtained by collaborators. The base, before pigments are added, is completely soluble in warm chloroform. The method described gives theoretical results for triethanolamine and for total acid when applied to the base. It is only after the base has been heated and milled with bone black that the method begins to give less than theoretical results.

It is recommended* that further work be done to improve the accuracy of this method, and that work be continued with eyebrow pencils and eye shadows.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 50 (1949).

The Associate Referee wishes to acknowledge the kind cooperation of the following collaborators: H. Kohnstamm & Co., W. C. Bainbridge; Kolmar Laboratories, H. Heinrich; Avon Allied Products, Inc., J. M. Williams; Max Factor & Co., London, Frank Atkins; Max Factor & Co., John Hall.

REPORT ON COSMETIC POWDERS

By GEORGE McCLELLAN (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

A face powder mixture of known constitution was subjected to collaborative study. The method of analysis specified was essentially that published by the author in *This Journal*, 25, 909 (1942), but with so many revisions that, for purposes of clarity, complete reproduction seems necessary.

CONSTITUENTS OF FACE POWDER

Fats and Fatty Acids as Stearic Acid

Weigh about 2 g of the powder into glass-stoppered 250-ml Erlenmeyer flask. Add 30 ml of benzene and swirl to mix thoroly. Add 10 ml of HCl, and swirl, removing stopper frequently to allow escape of CO₂ from carbonates. When pressure has spent itself, add 50 ml of petroleum ether, and shake cautiously with periodic removal of stopper until pressure again subsides. Then shake vigorously about 50 times. Decant ether layer thru a pledget of cotton into flask containing a few glass beads, that has been weighed with a similar flask as a counterpoise. (This decantation involves no danger of loss, for the particles of powder are tenaciously retained in the acid layer.) Again add 50 ml of petroleum ether and repeat shaking and decantation. Repeat with a third 50-ml portion of petroleum ether. Evaporate to dryness on steam bath under hood. Place in a draft oven at 100 degrees C. for one hour, heating flask used as a counterpoise at the same time. Remove flasks, cool, and weigh as stearic acid.

Total Zinc

REAGENTS

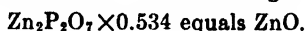
(a) *Wulfig's precipitant*.—Dissolve 80 g of finely ground ammonium carbonate in a mixture of 90 ml of NH₄OH and 375 ml of H₂O, and add 475 ml of 95% ethyl alcohol, which may or may not cause precipitation, depending on the temperature. Let any precipitate settle, and use supernatant soln.

(b) *Wash soln*.—Mix equal volumes of Wulfig's precipitant and 95% ethyl alcohol.

DETERMINATION

Weigh ca 2 g of the powder into a platinum dish and ignite to light gray ash at 600–650°C. Do not heat longer than necessary. With the aid of a wide-mouth funnel, transfer ash to a 500-ml glass-stoppered Erlenmeyer flask. Add 100 ml of Wulfig's precipitant in such manner as to wash down funnel. Stopper flask, and shake vigorously for 1 min., pausing from time to time to remove stopper and relieve pressure. Let sit overnight. Filter contents thru 12½ cm medium quantitative paper. With wash soln from a wash bottle, wash out flask, pouring washings thru filter; but make no attempt completely to transfer residue. Reserve flask for later deter-

mination of acid-soluble constituents. Wash residue on paper thoroly with wash soln. Determine zinc in filtrate as follows: Exactly neutralize to methyl red with HCl, add 200 ml of H₂O, and bring nearly to boiling on hot plate. Add 60 ml of 10% (NH₄)₂HPO₄, and continue to heat at just below boiling for 30 min. Remove and allow to cool slowly to room temp. Filter thru Gooch crucible that has been tared after ignition for 10 min. at full heat of Fisher burner. Wash with freshly prepared 1% soln of (NH₄)₂HPO₄, and finally with 50 ml of 50% alcohol. Discard filtrate. Place Gooch crucible in porcelain crucible of suitable size, and dry over low flame. Increase temp. and ignite at full heat to constant weight.



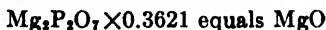
(Talcum powders often contain boric acid. When present, it will accompany ZnO, and must be separated before precipitation of the zinc.)

Acid-Soluble Calcium

Place paper containing residue from the zinc separation in a platinum dish and burn off paper at below 650°C. Transfer to a 250-ml beaker. Use 100 ml of (1+9) HCl to wash residue out of flask used in zinc separation, adding washings to beaker. (If some residue still clings to inside of flask, tilt up at steep angle over beaker, and wash out with stream of water from wash bottle.) Stir thoroly, allow to sit for 10 min. and filter thru medium quantitative paper. Disregard turbidity in filtrate, since this will be recovered in the next step. Wash residue on paper three times with distilled water. Place in a platinum dish not less than 6 cm in diam. nor less than 2 cm high, and hold pending addition of recovered acid-soluble Fe, Al, and BaSO₄. With NH₄OH, nearly neutralize the filtrate to methyl red. Add 200 mg of (NH₄)₂SO₄ and enough Br₂ water to destroy indicator and distinctly color soln. Boil free of bromine, add more methyl red, and while still nearly boiling add NH₄OH dropwise to the first distinct yellow, avoiding any excess. Let sit for ca 3 min., and filter thru a medium quantitative paper. Wash with hot 2% soln of NH₄Cl. Transfer paper and residue to the platinum dish containing acid-insoluble constituents. Determine calcium in filtrate according to 6.48, beginning with "heat to boiling. . . ."

Acid-Soluble Magnesium

Determine in filtrate from acid-soluble calcium as directed in 37.64 (p. 642).



Decomposition of Silicates; Solution of Titanium; Estimation of Barium Sulfate

APPARATUS

Air bath.—On a tripod over Fisher burner place clay triangle having a per side length of about 3 inches. In this triangle set a nickel or iron crucible of about 125 ml capacity, and on top of the crucible set a second clay triangle having a per side length of about 2½ inches. Purpose is to supply controlled radiated heat to platinum dish resting on the top triangle.

METHOD

Ash residues reserved in platinum dish (acid-insoluble portion and materials recovered prior to calcium precipitation) at below 650°C. Pulverize ash with flattened glass rod, and moisten with 4 ml of water. Add 4 ml of H₂SO₄, place under a hood, and fill dish to ca one-fourth of its depth with HF (48%). Evaporate on the air bath, swirling occasionally to mix contents, until only the H₂SO₄ appears to remain; then cautiously heat over the low flame of a Fisher burner to a pasty consistency. (Do not take to complete dryness.) Add 15 g of pulverized potassium pyrosulfate, and heat to melting. Continue heating, gradually raising temp. until a clear melt is ob-

tained. This will be achieved only when the dish glows red-hot and the melt orange-red. Too rapid heating will cause spattering. Foaming will occur but is not to be feared. At the completion of fusion, the clarity of the melt may be marred by bubbles and possibly by a few flakes of K_2SO_4 produced by the high temp., but these may be disregarded if the melt is generally clear. Set dish aside on an asbestos board and allow to cool. Melt will normally crack away from dish during cooling. Dislodge melt into a 600-ml beaker, wash dish with successive portions of hot (1+19) H_2SO_4 until a volume of about 150 ml is obtained, and boil until the melt goes into soln. If present, $BaSO_4$ comes down at this point. In this event, let digest on a steam bath for one hour, allow to cool, dilute to about 400 ml, stir well, and allow to sit for at least two more hours. Filter thru the finest available quantitative paper, catching filtrate in a 500-ml volumetric flask. Wash thoroly three times with water. Transfer residue to a tared porcelain crucible, burn off paper at low temp., and ignite at dull red heat. Weigh as $BaSO_4$. (Residues amounting to less than 0.5% should not be counted as $BaSO_4$. Where they occur, they represent HF-resistant silicate or quartz originally present in the talc or kaolin.)

Total Titanium and Iron

APPARATUS

Jones reductor.—Take a 50-ml pinchcock buret (without pinchcock attachment), and with a long glass rod ram down into its constricted lower end a pledget of glass wool. Fill buret to about the 15 ml mark with 20- or 30-mesh amalgamated Zn. (Zn may be amalgamated by letting fall into 200 ml of H_2O containing 4 g of dissolved $HgCl_2$ and 10 ml of H_2SO_4 . It should be washed several times with distilled H_2O by decantation before being put into buret.) Fit constricted lower end of buret with a 4-inch piece of thick-walled rubber tubing bearing a screw-clamp about the middle and terminating in a glass tube thrust thru a one-hole #7 rubber stopper. The stopper should be fitted to a 500-ml vacuum flask and the glass tube should be of such length as to reach within about 2 inches of the bottom of the flask. When not in use, the Jones reductor should be kept filled with distilled water.

DETERMINATION

Make filtrate from $BaSO_4$ to volume, pipet into a beaker an aliquot of 100 ml, and add with stirring 5 ml of H_2SO_4 . Place in vacuum flask 10 ml of 10% ferric alum (free of ferrous Fe and other substances reducing $KMnO_4$). Fit flask to reductor, apply vacuum, and open screw clamp enough to permit controlled passage of liquid into flask. When meniscus in buret has sunk nearly to level of zinc, add more soln. (It is preferable never to expose amalgamated zinc to the air.) When all of soln has been added, add in the same manner about 100 ml of distilled H_2O . Close screw clamp just before meniscus of last washing reaches level of zinc, release vacuum, and disconnect flask. Transfer contents to 300-ml tall-form beaker and add 3 ml of syrupy phosphoric acid. Using a 10 ml microburet, titrate over a white surface with 0.1 N $KMnO_4$ to the first pink. Make up a blank containing 3 g of potassium pyrosulfate and 6.5 ml of H_2SO_4 in 100 ml of distilled H_2O . Put this thru identically the same treatment the sample received, finally titrating to the same shade of pink. Subtract titre of blank from that of sample. Corrected titre $\times .008$ equals total (TiO_2 plus Fe_2O_3) (these have practically the same equivalent weight).

Total Iron

REAGENT

Titanium trichloride, 0.05 N $TiCl_3$.—Make up according to directions in 21.36 (p. 290), but containing only half as much $TiCl_3$ as required for the 0.1 N soln.

Standardize according to either of the methods listed under 21.37 except that standardization should be conducted using an ordinary micro-buret and titrating into an open beaker. The soln should be kept in an ordinary glass-stoppered bottle and re-standardized immediately before each set of determinations.

DETERMINATION

Pipet an aliquot of 100 ml from volumetric flask into a 150-ml beaker. Add 1 g NH_4CNS . Slowly and with thoro stirring, titrate with 0.05 N TiCl_3 from a micro-buret to disappearance of the red color. Run a blank on 3 g of potassium pyrosulfate and 6.5 ml H_2SO_4 in 100 ml of distilled H_2O . (Blank is often nil.) Corrected titer $\times .004$ equals Fe_2O_3 .

Total Titanium

Per cent total (TiO_2 plus Fe_2O_3)—per cent total Fe_2O_3 equals per cent total TiO_2 .

Total Oxides of Iron, Titanium, and Aluminum

Pipet an aliquot of 250 ml from volumetric flask into a 600-ml beaker. Add a few drops of methyl red indicator and 5 g of NH_4Cl , and bring to boil. Neutralize by adding NH_4OH dropwise just to the first distinct yellow. Let sit for about 3 min., and filter thru a $12\frac{1}{2}$ cm medium quantitative paper. Wash several times with hot 2% NH_4Cl . Place paper in a tared crucible and dry in an oven or an air bath. Transfer to a muffle furnace at room temp., and raise heat to about 1100 degrees. Ignite to constant weight. Result is total (Al_2O_3 plus Fe_2O_3 plus TiO_2).

Total Aluminum

Per cent total (Al_2O_3 plus Fe_2O_3 plus TiO_2)—per cent total (Fe_2O_3 plus TiO_2) equals per cent total Al_2O_3 .

Acid-Insoluble Calcium

Determine calcium in the filtrate from the ammonium hydroxide precipitate according to directions in 6.48 (p. 66), beginning with "heat to boiling. . ."

Acid-Insoluble Magnesium

Determine in filtrate from acid-insoluble calcium by 37.64 (p. 642). $\text{Mg}_2\text{P}_2\text{O}_7 \times 0.3621$ equals MgO .

Silica

Weigh about 1 g of the powder into a 250-ml beaker. Moisten with alcohol and add 100 ml of (1+9) HCl . Stir, and allow to stand for 10 min. Filter thru $12\frac{1}{2}$ cm medium quantitative paper. Wash the residue 3 times with H_2O . Transfer paper to a platinum crucible and ash at below 650°C . Cool, and pulverize ash with a flattened glass rod. Add 6 g of Na_2CO_3 , a portion at a time, intimately mixing with the same glass rod between additions. Use the last of the Na_2CO_3 to sprinkle over the top of the mixture. Place in a muffle furnace at below 800°C ., and raise temp. to bring contents into fusion. Heat at ca 1000°C . for 15 min. Remove the crucible and let cool. Dislodge the melt into a dry 500-ml beaker. (Dislodging the melt is not always easy. It often helps to return the crucible to the hot furnace for $\frac{1}{2}$ min., then remove it and immediately dip about two-thirds of its length in a beaker of H_2O . If repeated a sufficient number of times, this treatment causes the melt to crack away from the platinum so that it can be removed by simply upending the crucible over the beaker.) In a graduate mix 15 ml of HNO_3 with 5 ml of H_2O , and wash the crucible with small successive portions of the mixture, adding washings to the beaker. If soln of the melt becomes slow, hasten its disintegration by gentle pressure with a glass rod.

When the Na_2CO_3 in the melt has dissolved, place the beaker under a hood and add, in the order named, 5 g of NH_4Cl and 25 ml of HClO_4 (60%). Cover the beaker with a watch glass, and boil over a moderate flame until oxides of nitrogen have passed off and the HClO_4 refluxes down the sides of the beaker. Cool the mixture slightly, add 150 ml of very hot water, stir, and let sit until silica settles to the bottom. Decant supernatant liquid thru a 12½ cm medium quantitative paper, and transfer residue to paper using hot water and policing out beaker. Wash thoroly five times with hot water. Transfer to a platinum dish, burn off paper, and ignite to constant weight at about 1100°C. Weigh as crude silica. To the residue in the dish add ca 2 ml of (1+9) H_2SO_4 and enough HF (48%) to cover the silica. Heat on a steam bath under the hood until silica and excess HF have passed off. Cautiously heat over the non-reducing flame of a Fisher burner until fumes of SO_3 have ceased to be evolved, and then heat strongly for several min. Cool and reweigh. The difference between this weight and the weight of crude silica is weight of SiO_2 .

Starch

Weigh ca 5 g of the powder into a 500-ml Florence flask (preferably standard taper). Moisten with 10 ml of alcohol. Acid-wash according to directions in 17.20 (p. 212), hydrolyze starch as directed under 27.33 (but filter hydrolyzed mixture before and not after making to volume), and determine dextrose by 34.39 and 34.40 (p. 572).

COLLABORATIVE

A collaborative face powder mixture was prepared from nine components, all previously assayed for all constituents except carbon dioxide and water. The Associate Referee was able to secure only two collaborators—Sylvan H. Newburger and Charles Graichen, both of the Cosmetic Division of the Food and Drug Administration, Washington, D. C. These gentlemen deserve much credit for bringing to a successful conclusion a most involved and time-consuming piece of analysis.

TABLE 1.—*Collaborative results*
 CO_2 and H_2O not determined

CONSTITUENT	PRESENT	GRAICHEN		MCCLELLAN		NEWBURGER	
		1	2	1	2	1	2
Stearate as Stearic Acid	4.5	4.6	5.0	4.6	4.6	4.5	4.5
Total ZnO	10.0	9.9	9.9	10.1	9.9	9.8	9.9
Acid-soluble CaO	3.1	3.2	3.2	3.0	3.0	3.0	3.0
Acid-soluble MgO	2.5	2.4	2.4	2.5	2.5	2.5	2.5
BaSO ₄	9.9	9.8	9.9	9.6	9.7	9.7	9.7
Total Fe ₂ O ₃	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total TiO ₂	5.0	4.7	5.0	4.8	4.9	4.8	4.7
Total Al ₂ O ₃	8.0	7.9	7.8	8.3	8.0	8.4	8.3
Acid-insoluble MgO	8.0	8.2	8.2	8.3	8.2	8.2	8.2
Acid-insoluble CaO	2.1	2.0	2.1	1.8	1.8	2.0	2.0
SiO ₂	26.8	25.8	25.6	26.6	27.1	26.1	26.1
Starch	8.8	9.3	9.3	8.9	8.9	9.5	9.4
Total	88.9	88.0	88.6	88.7	88.8	88.7	88.5

<i>Component</i>	<i>Per cent by weight in mixture</i>
Talc	30
Kaolin	20
Zinc oxide	10
Starch	10
Barium sulfate	10
Titanium dioxide	5
Calcium carbonate	5
Magnesium carbonate	5
Magnesium stearate	5

RECOMMENDATIONS*

It is recommended—

(1) That the above method for face powders be adopted as official, first action.

(2) That this subject be continued.

REPORT ON COSMETIC SKIN LOTIONS

By HENRY R. BOND (Food and Drug Administration, Federal Security Agency, Kansas City, Mo.), *Associate Referee*

DETERMINATION OF GLYCEROL, ETHYLENE GLYCOL, AND PROPYLENE GLYCOL IN A CLEAR TYPE SKIN LOTION

As one phase of the study of cosmetic skin lotions, the clear type lotion was selected for the purpose of devising suitable assays for as many as possible of the common ingredients involved.

Glycerol, in particular, and two of the glycols, ethylene and propylene, are relatively common constituents of many skin lotions. It was decided to utilize a method for determining quantitatively each of the three in a mixture. Such a method had been devised by Irwin S. Shupe¹ under the title "Periodate Reaction Applied to Cosmetic Ingredients."

A slightly modified form of this method was employed for the assay of the samples prepared for collaborative study. Collaborators were also requested to determine the glycerol content of the samples by the method of Newburger and Bruening² for the purpose of comparing results obtained through the use of brom-cresol-purple indicator as opposed to methyl red.

Since the periodate volumetric method employed was semi-micro in nature, the collaborative samples contained only small amounts (1 gram or less) of the constituents to be determined. The solutions were slightly acetic and the two samples sent to each collaborator were identical in composition, in that each was made from one and the same stock solution

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 80 (1949).

¹ *This Journal*, 26, 249 (1943).

² *Ibid.*, 30, 651 (1947).

with the same degree of dilution. Therefore, results obtained from the two samples should have been practically identical.

The results of collaborative study are tabulated below in an abridged form, merely stating the average percentage recovery obtained by each collaborator.

TABLE 1.—*Collaborative results*

CONSTITUENT	MILLIGRAMS OF CONSTITUENT PER 20 ML OF SAMPLE	COLLABORATORS' PER CENT RECOVERY			
		A	B	C	D
(1) Glycerol (using methyl red)	18.65	97.59	94.91	—	108.71
(2) Glycerol (using brom-cresol- purple)		99.81	91.02	97.05	—
Propylene glycol	16.28	98.43	94.60	100.12	85.78
Ethylene glycol	13.00				
Calc. using (1) above.		100.54	107.67	—	99.32
Calc. using (2) above		97.56	109.27	98.85	—

While collaborators A and C obtained results which could be considered excellent, it is believed that the method should be subjected to further collaborative study before its acceptance is recommended. A comparison of recoveries of glycerol, using two different indicators, indicates brom-cresol-purple as the better.

RECOMMENDATION*

It is recommended that a new series of samples be subjected to more extensive collaborative study in order to determine the suitability of the method.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation of the efforts of the following members of the Food and Drug Administration: H. P. Bennett, New Orleans, and F. E. Yarnall, Kansas City, for aid in preliminary work; and G. E. Keppel, Minneapolis; S. T. Colamaria, Boston; W. S. Cox, Atlanta, and D. Banes, Chicago, as collaborators.

No reports were given on alkalies in cuticle removers, hair straighteners, cosmetic creams, deodorants and anti-perspirants, depilatories, hair dyes and rinses, mercury salts in cosmetics, or moisture in cosmetics.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 50 (1949).

The paper entitled "Analysis of Castor Oil in Lipstick," by S. H. Newburger, is published in *This Journal*, p. 658.

REPORT ON COAL-TAR COLORS

By G. ROBERT CLARK (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

RECOMMENDATIONS*

Acetates, Carbonates, Halides, and Sulfates in Coal-Tar Colors.—The Referee concurs in the recommendation for continuation of this topic. The Referee further recommends reassignment of this topic, as requested by the Associate Referee.

Buffers and Solvents in Titanium Trichloride Titration.—The Referee recommends continuation of this topic.

Ether Extract in Coal-Tar Colors.—The Referee recommends continuation of this topic.

Halogens in Halogenated Fluoresceins.—The Referee concurs in the recommendation that the proposed methods for chlorine and for chlorine and bromine, when both are present, be studied.

Identification of Coal-Tar Colors.—The Referee recommends continuation of this topic, and that it be reassigned.

Volatile Amine Intermediates.—The Referee concurs in the recommendation for continuation of this topic.

Non-volatile Unsulfonated Amine Intermediates in Coal-Tar Colors.—The Referee concurs in the recommendation for continuation of this topic.

Sulfonated Amine Intermediates in Coal-Tar Colors.—The Referee recommends continuation of this topic.

Unsulfonated Phenolic Intermediates in Coal-Tar Colors.—The Referee concurs in the recommendation that the topic be continued.

Sulfonated Phenolic Intermediates in Coal-Tar Colors.—The Referee recommends continuation of this topic.

Intermediates Derived from Phthalic Acid.—The Referee recommends continuation of this topic.

Mixtures of Coal-Tar Colors for Drug and Cosmetic Use.—The Referee recommends continuation of this topic.

Pure Dye in Lakes and Pigments.—The Referee concurs in the recommendation—

(1) That the proposed method for the determination of pure dye in D&C Reds Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 31, and 34, and Ext. D&C Red No. 2, be adopted as official, first action.

(2) That the topic be continued.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 50 (1949).

The Referee further recommends that the topic be reassigned.

Spectrophotometric Testing of Coal-Tar Colors.—The Referee concurs in the recommendation that the topic be continued.

The Referee further recommends that the topic be reassigned.

Subsidiary Dyes in D&C Colors.—The Referee recommends continuation of this topic.

Subsidiary Dyes in FD&C Colors.—The Referee concurs in the recommendation that the topic be continued.

Hygroscopic Properties of Coal-Tar Colors.—The Referee recommends continuation of this topic.

Lead in Coal-Tar Colors.—The Referee concurs in the recommendation that the method, described by N. Ettelstein in a contributed paper (*This Journal*, 30, 552 (1947) and supported by collaborative results, presented at the 1947 meeting of the Association, be adopted as official, first action, for the determination of lead in all straight coal-tar colors listed as certifiable under the regulations promulgated in accordance with the Federal Food, Drug, and Cosmetic Act, except those containing calcium, barium, or strontium.

Lead in Lakes of Coal-Tar Colors.—The Referee recommends that the methods described by the Associate Referee be adopted as official, first action, based upon the collaborative results presented in the report for this year and the recovery experiments presented at the 1947 meeting. The Referee further recommends the addition of the following topics for study:

The Determination of Arsenic in Coal-Tar Colors.

The Determination of Heavy Metals in Coal-Tar Colors.

The Boiling Range of Pseudocumidine and Xylidine in Certified Coal-Tar Colors.

REPORT ON HALOGENS IN HALOGENATED FLUORESCEINS

By N. GORDON (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

CHLORINE

The work covered by this report consists primarily of a collaborative study of the determination of chlorine and bromine by the method proposed by Clark and Jones (1). The method for determining bromine, 21.53, is official, first action (*Methods of Analysis*, 6th Ed.). A previous collaborative study (2) of the method as applied to samples containing both bromine and chlorine did not give completely satisfactory results.

It was felt that the inconsistent results obtained in last year's study may have been due in part to difficulties in the decomposition step in the

analysis. It was decided, therefore, to submit samples that would permit an evaluation of each part of the procedure.

A sample of purified potassium bromide was submitted as a further check on the bromine titration and to familiarize the collaborators with the titration.

A solution containing potassium bromide, sodium chloride, and hydrazine sulfate was submitted to check the accuracy of the steps subsequent to the decomposition of the sample. The contents of this solution were not disclosed to the collaborators.

As a check on the reliability of the decomposition step a sample of a commercial preparation of D&C Red No. 24 was submitted for analysis.

Reports containing analytical data were received from the following collaborators:

Ansbacher-Siegle Corporation—H. Holtzman reporting.

Max Factor, Inc.—H. R. Cohen reporting.

H. Kohnstamm and Company, Inc.—Mrs. V. Schmuckli reporting.

National Aniline Division, Allied Chemical & Dye Corp.—A. T. Schramm reporting.

Cosmetic Division, Food and Drug Administration—J. H. Jones and L. S. Harrow reporting.

One collaborator reported considerable difficulty in obtaining reproducible results in the bromine determination.

Another collaborator suggests the rate at which the sodium thiosulfate solution is added during the titration may be a factor in the accuracy of the results; low results were attributed to a slow rate of addition in one instance and a rapid rate was recommended.

Difficulty was experienced with the digestion by one collaborator who reported that "There were sudden ebullitions that might account for the wide discrepancy in the findings for the chlorine determination."

All reported results are shown in the order that they were received, in Tables 1-3.

With one exception, the results of the collaborators on the purified potassium bromide are in good agreement with the theoretical value. The average results for these collaborators is 99.8 per cent of the theory; the highest, 100.1; and the lowest, 98.5.

The results of six of the seven collaborators on the solution containing both bromine and chlorine are in good agreement with one another and the calculated values. The maximum error in the values obtained by these collaborators is less than 1 per cent for either the bromine or chlorine determination. The other collaborator reported inconsistent results on the potassium bromide sample. It would appear, therefore, that the proposed method is capable of giving accurate results on solutions containing both bromine and chlorine.

The results for chlorine in D&C Red No. 24 are somewhat variable. The

TABLE 1.—*Collaborative results for bromine in purified KBr*

COLLABORATOR	BROMINE	COLLABORATOR	BROMINE
	<i>per cent</i>		<i>per cent</i>
Associate Referee	67.0	5	66.15
	66.2		66.15
	66.3		66.35
	66.4		66.73
	66.7		
2	67.07	6	66.8
	66.96		66.8
	67.19	7	67.0
3	57.0–77.0*	Average.....	66.8
4	67.03	Average Deviation..	0.23
	67.23	Theoretical.....	67.15
	67.23		

* Not included in average.

TABLE 2.—*Collaborative results for bromine and chlorine in a solution of purified KBr and NaCl*

COLLABORATOR	BROMINE	CHLORINE
	<i>grams per 10 ml</i>	<i>grams per 10 ml</i>
Associate Referee	0.0439	0.0214
	0.0439	0.0213
	0.0439	0.0213
2	0.04416	0.02142
	0.04426	0.02132
	0.04416	0.02135
3	0.0954	0.0232
	0.0949	0.0234
4	0.0443	0.0212
	0.0443	0.0212
	0.0443	
5	0.0440	0.0212
	0.0444	0.0214
	0.0440	0.0210
	0.0444	
6	0.0437	0.0215
	0.0438	0.0216
	0.0438	
	0.0439	
7	0.0439	0.0212
Calculated	0.0440	0.0214

TABLE 3.—*Collaborative results for chlorine in D&C Red No. 24**

COLLABORATOR	CHLORINE	COLLABORATOR	CHLORINE
	<i>per cent</i>		<i>per cent</i>
Associate Referee	29.2	5	31.14
	29.2		30.24
			29.96
2	29.35	6	29.5
	29.35		29.4
	29.26		29.5
3	28.6	7	28.7
	28.7		28.7
			28.9
4	29.15	Average	29.29
	29.02		
	29.19	Average Deviation	0.4 or 1.4%
	29.05		of the average

* The sample submitted was a commercial sample; a pure sample of D&C Red No. 24 would contain 30.2% Cl.

average deviation from the mean for the seven collaborators is 1.4 per cent. If the set of high results is omitted, the average deviation from the mean is less than 1 per cent. It would appear from these data that the decomposition procedure is capable of giving consistent results.

It seems reasonable to assume, therefore, that the proposed methods for bromine and/or chlorine in halogenated fluoresceins will give satisfactory results if the directions are carefully followed.

RECOMMENDATIONS*

It is recommended—

- (1) That the chlorine method be adopted as official.
- (2) That the method for bromine and chlorine, when both are present, be adopted as official.
- (3) That the topic be continued, to study more rapid methods and to provide an official method for iodine.

REFERENCES

- (1) CLARK, G. R., and JONES, J. H., "Determination of Chlorine and Bromine," *This Journal*, **26**, 433 (1943).
- (2) GORDON, N., "Report on Halogens in Halogenated Fluoresceins," *This Journal*, **31**, 589 (1948).

* For report of Subcommittee B and action of the Association, see *This Journal*, **32**, 50 (1949).

VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS

BY ALICE B. CAEMMERER (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.),
Associate Referee

At the annual meeting of the A.O.A.C. in October 1947, a method¹ for the determination of volatile amine intermediates in coal-tar colors was reported. This method involved the isolation of the amine by steam distillation, diazotizing the amine and coupling with 1-(4 sulfophenyl)-3-methyl-5-pyrazolone. The resulting dye was then titrated with standard titanium trichloride solution and the weight of the amine calculated. This study has been extended to the determination of *p*-toluidine and *o*-toluidine in various certifiable colors. The results obtained are shown in Table 1.

TABLE 1.—*Recoveries of added intermediates in analyses by Associate Referee*

COLOR	RECOVERY
	<i>per cent</i>
Para-toluidine	
D&C Violet No. 2	96.5
D&C Blue No. 5	90.0
D&C Green No. 5	97.8
D&C Green No. 6	96.0
Average	95.0
Ortho-toluidine	
FD&C Orange No. 2	96.8

Since the recovery of *p*-toluidine and *o*-toluidine was good, it was decided to submit samples containing known amounts of each intermediate to collaborative study. Accordingly, samples of D&C Green No. 5 and FD&C Orange No. 2, each containing 20 mg of *p*-toluidine and *o*-toluidine, respectively, were submitted to various collaborators. The results are shown in Table 2.

From an inspection of Table 2 it is obvious that either the method is unworkable or that some difficulty was encountered with the samples. Since the Associate Referee has obtained good recoveries on known amounts of intermediate, it is thought probable that the difficulty lies in the sample. These samples were sealed in long tubes, hence it may be that all of the sample was not transferred to the distillation apparatus. Therefore, it is proposed that a new sampling procedure be devised and that the method be resubmitted to collaborative study.

¹ *This Journal*, 31, 582 (1948).

TABLE 2.—*Collaborative studies of recovery of o-toluidine and p-toluidine 0.2% added to each sample*

COLLABORATOR	D&C GREEN NO. 5 p-TOLUIDINE	FD&C ORANGE NO. 2 o-TOLUIDINE
	<i>per cent</i>	<i>per cent</i>
1	0.141	0.160
	0.144	0.150
2	0.166	0.148
	0.875	0.193
3	0.1051	0.1994
		0.1956
4	0.19	0.16
	0.18	0.15

The Associate Referee wishes to thank the following collaborators for their assistance in this work:

Ansbacher-Siegle Corporation—H. Holtzman reporting.

H. Kohnstamm and Company, Inc.—Louis Koch reporting.

Wm. J. Stange Company—W. H. Kretlow reporting.

It is recommended* that the topic "Volatile Amine Intermediates in Coal-Tar Colors" be continued.

REPORT ON ACETATES, CARBONATES, HALIDES, AND SULPHATES IN CERTIFIED COAL-TAR COLORS

SODIUM ACETATE IN FD&C BLUE NO. 1

By J. SCHIFFERLI and A. T. SCHRAMM (National Aniline Division, Allied Chemical and Dye Corporation, Buffalo, N. Y.), *Associate Referee*

The Coal-Tar Color Regulations stipulate that the sodium acetate content of FD&C Blue No. 1 must not exceed 3.0 per cent.

The tentative A.O.A.C. method¹ for the determination of this impurity in FD&C Blue No. 1 has been found to be time-consuming and productive of results lacking in precision and accuracy. Sclar and Clark described a method² based on Freudenberg's method³ for the determination of acetyl groups. This method, however, did not progress to the desired state of collaborative work. The principle of the method of Sclar and Clark involves esterification of sodium acetate to ethyl acetate in the presence of

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 51 (1949).

¹ *Methods of Analysis*, A.O.A.C., 1945, 286.

² Sclar, R. N., and Clark, G. R., *This Journal*, 27, 472 (1944).

³ Freudenberg, K., and Harder, M., *Ann.*, 433, 230 (1923); 494, 68 (1932).

p-toluenesulfonic acid and silver toluenesulfonate, distillation, and saponification of the distilled ester with a measured excess of standard sodium hydroxide.

The present authors' investigation revealed that, although the method of Sclar and Clark is satisfactory in principle, it is subject to improvement by more careful definition of procedural detail. To effect the desired improvement, the following modifications have been made:

1. Sample weight increased from 2 grams to 5 grams;
2. Meta-cresol purple substituted for phenolphthalein as indicator to obtain the true equivalence point;
3. Hydrolysis of the ester protected with a tube containing carbon dioxide absorbing material;
4. Distillation time reduced by wrapping the Kjeldahl trap with insulating material;
5. Distillation time further reduced by addition of anti-bumping agent to the flask and use of an Allihn condenser;
6. Anhydrous *p*-toluenesulfonic acid used to favor the ester formation;
7. Sequence of addition of reagents changed to facilitate wetting of the dye and the *p*-toluenesulfonic acid.

METHOD

REAGENTS

p-Toluenesulfonic acid.—Dry *p*-toluenesulfonic acid monohydrate overnight at 110°C., cool, and grind to a powder.

Silver toluenesulfonate.—Dissolve reagent silver oxide or carbonate in about 10% excess of *p*-toluenesulfonic acid solution, evaporate to dryness, and dry at 135°C. for 8 hours.

APPARATUS

The apparatus can be assembled from stock items. The distilling flask has a capacity of 100–125 ml and is provided with a Kjeldahl trap which is wrapped with asbestos rope or other heat insulator to increase the distillation rate. The assembled apparatus is shown in Figure 1.

PROCEDURE

To a 500 ml Erlenmeyer flask add 100 ml of distilled water, a drop of *m*-cresol purple indicator soln (0.5 g of solid indicator triturated with 13 ml of 0.1 *N* NaOH and diluted with distilled water to make 100 ml.) and sufficient 0.1 *N* NaOH or 0.1 *N* HCl to turn the color of the soln just yellow. Place the flask and contents under the condenser.

Transfer 30 ml of anhydrous alcohol to the distillation flask and add 5.00 g of the sample, 5 g of *p*-toluene-sulfonic acid, and 1 g of silver toluenesulfonate thru a powder funnel.

Add 3–4 pieces of alundum or other anti-bumping agent and mark the level of the liquid in the distillation flask. Wash the funnel and neck of the flask with 25 ml of anhydrous alcohol. Shake the flask to mix the contents thoroly and attach it to the condenser.

Immerse the distillation flask as far as possible in a beaker of hot water and heat the water to boiling. After about 25 ml of distillate has collected, remove the heat

source and slowly add 25 ml of anhydrous alcohol to the distillation flask. When it again begins to distill quietly replace the heat source until a second 25 ml of distillate has collected. Make a third addition and distillation in similar manner. Finally boil until the distillation rate is slow (about one-half hour total distillation time figured from the beginning of the first distillation).

Wash down the condenser with 50 ml. of distilled water into the receiver, and add to the receiver contents 50.0 ml of 0.1 *N* NaOH. Add 3-4 pieces of alundum and

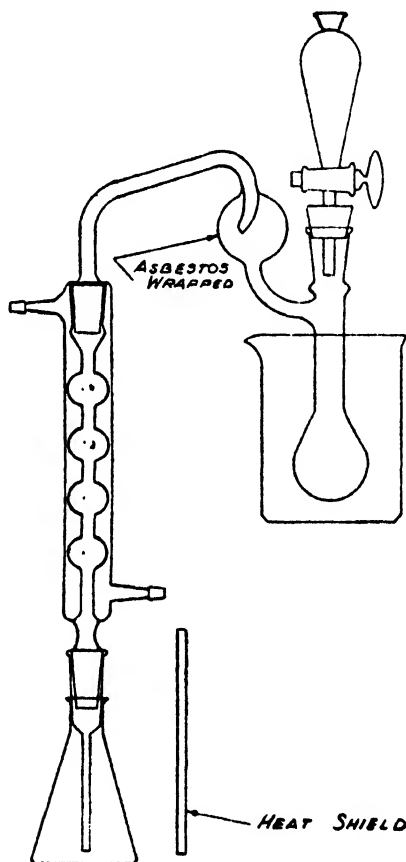


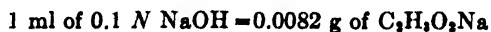
FIG. 1

connect to a reflux condenser fitted with an absorption tube containing ascarite or other carbon dioxide absorbing material.

Reflux for 10 min. Cool to room temp., add a few drops of *m*-cresol purple indicator, and titrate with 0.1 *N* HCl to the yellow-green color which does not change in hue on further addition of acid.

Determine the blank by duplicating the procedure with the omission of the sample.

Calculate the sodium acetate from the net volume of standard NaOH soln required.



Results by this method are shown in Table 1. Several determinations of the blank were run on the reagents used, including various supplies of anhydrous alcohol. The blank ranged from 0.3 to 0.5 ml of 0.1 N HCl corresponding to 0.05 to 0.08 per cent of sodium acetate in a 5 gram sample.

TABLE 1.—*Determination of sodium acetate in FD&C Blue No. 1*

BATCH	ADDED	FOUND*	CALCULATED TOTAL	DIFFERENCE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
A	0	0.08		+0.01†
A	0	0.05	---	-0.02†
B	0	1.62		+0.01†
B	0	1.59		-0.02†
C	0	2.95	---	-0.01†
C	0	2.96		0.00†
C	0	2.96	---	0.00†
A	2.81	2.95	2.88	+0.07
A	2.81	2.92	2.88	+0.04
A	2.81	2.94	2.88	+0.06
A	4.00	4.04	4.07	-0.03
A	4.00	4.08	4.07	+0.01
A	6.00	6.01	6.07	-0.06
A	10.00	9.93	10.07	-0.14

* Deductions for reagent blanks were made

† This figure is the difference from the average of the replicate determinations on the batch.

An average difference of 0.01 per cent between a single determination and the average of replicate determinations was obtained in seven determinations involving three batches of FD&C Blue No. 1. An average difference of -0.007 per cent was obtained between the found and calculated values in seven determinations of the recovery of added sodium acetate.

SUMMARY

A method for determining sodium acetate in FD&C Blue No. 1 has been described. Typical results are given.

REPORT ON UNSULPHONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS

BETA-NAPHTHOL IN D&C RED NO. 35

By H. HOLTZMAN (*Associate Referee*) and H. GRAHAM (*Ansbacher-Siegle Corporation, Rosebank, Staten Island, New York*)

This is the initial report of a series on the determination of unsulphonated phenolic intermediates in coal-tar colors.

EXPERIMENTAL

Pure beta-naphthol was obtained by recrystallization from toluene and the crystals washed with petroleum ether (7).

A purified D&C Red No. 35 was prepared by successively washing with dilute caustic soda, followed by dilute acid and water washes (8).

The following method of analysis was evolved:

PROCEDURE

Transfer a 10 g sample to a 250 ml beaker. Add 50 ml of 1:10 HCl. Heat to 50°. Stir on automatic agitator (ca 120 r.p.m.), for 10 min. Filter. Wash with ca 50 ml 50° 1:10 HCl. Return the filter paper and residue to original beaker. Add another 50 ml of 1:10 HCl. Repeat the heating, stirring, and filtering. Wash with 50° 1:10 HCl. The total filtrates (ca 200 ml) are transferred to a 500 ml wide-mouth Erlenmeyer flask. The pH is adjusted to neutrality with dilute NaOH (using phenolphthalein indicator). Add 10 gm Na Acetate. Cool to 5° in ice bath.

PREPARATION OF DIAZO

Make a soln of ca 0.05 *N* sulfanilic acid by dissolving 4.779 g of sulfanilic acid in 500 ml of H₂O, to which has been added 5 ml of 12 *N* HCl. Make a soln of ca 0.05 *N* NaNO₂ by dissolving 1.04 gm NaNO₂ in 300 ml H₂O. Place 40 ml of the sulfanilic acid in a 100 ml vol. flask. When cooled to 5° C, add 44 ml of the NaNO₂ soln and allow to diazotize. Test for excess nitrous acid and destroy excess with a few mg of sulfamic acid. Dilute to volume.

Add slowly 20 ml of diazo soln. Stir 5 min. and test for excess diazo with alk. beta naphthol soln. If not positive, add additional diazo until a positive test is obtained. Let stand for one hour.

Heat on water bath for $\frac{1}{2}$ hour to decompose excess diazo. Add 10 g sodium bitartrate which has been dissolved in 50 ml hot H₂O. Dilute with ca 100 ml ethyl alcohol. Titrate with TiCl₃ to a yellowish end point. (Back titrate with Methylene Blue, if desired.)

Calculations:

1 ml of 0.1 *N* TiCl₃ = .0036 g Beta Naphthol.

Dye coupling and analyses of known solutions of pure beta naphthol gave excellent recoveries, by the method given. Procedure was then applied to samples of the purified D&C Red No. 35, to which known amounts of beta-naphthol had been added, the latter in dilute alkaline solution.

COLLABORATIVE RESULTS

Samples of a commercial D&C Red No. 35 and a synthetically prepared sample containing 0.30% beta naphthol were submitted for collaborative analysis to P. T. Beeton and L. Krawer of Ansbacher-Siegle Corporation, and to C. Graichen of the Cosmetic Division of the Food and Drug Administration. Analytical results by the junior author are included.

It is of interest that in spite of the three divergent results, each collaborator duplicated his own results. On the basis of the foregoing, further work is required on the extraction procedure.

TABLE 1.—*Recovery of Beta Naphthol in sample of D&C Red No. 35*

BETA NAPHTHOL ADDED	DETERMINATION	TOTAL	BLANK	RECOVERY
<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.05	(1)	0.045	—	0.045
	(2)	0.049	—	0.049
0.10	(1)	0.098	—	0.098
	(2)	0.100	—	0.100
0.15	(1)	0.15	—	0.15
	(2)	0.13	—	0.13
0.25	(1)	0.24	—	0.24
	(2)	0.23	—	0.23
	(3)	0.23	—	0.23
0.30	(1)	0.41	0.12	0.29
	(2)	0.41	0.12	0.29
	(3)	0.35	0.09	0.26
	(4)	0.37	0.09	0.28
0.50	(1)	0.49	—	0.49
0.60	(1)	0.68	0.09	0.59
0.80	(1)	0.93	0.12	0.81
	(2)	0.91	0.12	0.79
	(3)	0.87	0.12	0.75

TABLE 2.—*Collaborative results*

COMMERCIAL D&C RED NO. 35			SYNTHETIC SAMPLE CONTAINING 0.3% (9)			
COLLABORATOR	DETERMINATION	BETA NAPHTHOL	COLLABORATOR	DETERMINATION	RESULT	(MINUS) (BLANK)
		<i>per cent</i>				
1	(1)	0.38	1	(1)	0.34	(0.28)
	(2)	0.36		(2)	0.30	(0.24)
2	(1)	0.35	2	(1)	0.34	(0.28)
	(2)	0.33		(2)	0.34	(0.29)
3*	(1)	0.67				
	(2)	0.68				
4	(1)	0.45				
	(2)	0.46				

* Collaborator No. 3 commented (a) that some fine particles of dye may have been carried through into the filtrate, as the methylene blue back titration end point faded on standing; (b) a greater volume of the dilute acid should be employed for each extraction, to improve efficiency of the extractions.

It is recommended* that this work be continued, to be followed by further collaborative study.

REFERENCES

- (1) *Allen's Commercial Org. Anal.*, Vol. II, p. 408.
- (2) *J. Soc. Chem. Ind.*, 16, p. 294.
- (3) *A.O.A.C. Bulletin* #107 (1907).
- (4) *Allen's Commercial Org. Anal.*, Vol. III, p. 209.
- (5) *Merck Index*.
- (6) *Allen's Commercial Org. Anal.*, Vol. III, p. 234.
- (7) *J. Soc. Chem. Ind.*, 16, p. 294.
- (8) Some of the batches of D&C Red No. 35 washed in this manner, gave a blank, which might be either residual beta naphthol or possibly dye or some decomposition product. This blank varied in amount in various batches, and is being investigated further, using D&C Red No. 35, recrystallized from chloroform.
- (9) The sample of D&C Red No. 35 gave an analytical blank of 0.06%, which was subtracted from the total result.

REPORT ON LEAD IN LAKES (ALUMINUM) OF COAL-TAR COLORS

By LEE S. HARROW (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the 1947 meeting of the A.O.A.C. the Associate Referee presented methods for the determination of lead in aluminum coal-tar color lakes and in calcium, barium, and strontium coal-tar color lakes.¹

Composite samples containing known amounts of lead were distributed to several laboratories for collaborative analysis by these methods. During the past year, reports were submitted by:

Ansbacher-Siegle Corporation—H. Holtzman reporting.

TABLE 1.—*Lead in D&C Blue No. 1, Aluminum Lake*

COLLABORATOR	LEAD FOUND	LEAD PRESENT
	p.p.m.	p.p.m.
1	23.5	23.6
	25.0	
	25.0	
	25.0	
2	73.0	76.6
	76.0	
3	23.0	23.6
	28.0	

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 51 (1949).

¹ *This Journal*, 31, 677 (1948).

H. Kohnstamm and Company, Inc.—I. Hanig reporting.

Cosmetic Division, Food and Drug Administration—C. Graichen reporting.

National Aniline Division, Allied Chemical & Dye Corp.—A. T. Schramm reporting.

The results submitted are shown in Tables 1 and 2.

TABLE 2.—Lead in D&C Red No. 9, Barium Lake

COLLABORATOR	LEAD FOUND	LEAD PRESENT
	p.p.m.	p.p.m.
1	75.0	76.0
2	35.0	36.0
	37.0	
	34.0	
	31.0	
3	56.7	56.0
	55.4	
	40.6	
4	51.0	56.0
	55.0	

Since so few collaborative reports were submitted, no recommendation of adoption of the method is made. It is recommended* that study on this topic be continued.

REPORT ON LEAD IN COAL-TAR COLORS

By NATHAN ETTTELSTEIN (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.),
Associate Referee

A study of a dithizone method for the determination of lead in coal-tar colors was presented at the 1946 meeting of the Association.¹ The method there described has been submitted to collaborative study. The results obtained are presented in Table 1.

The dithizone-lead determination under study consists of a preliminary digestion of the dye with nitric, sulfuric, and perchloric acids. The resulting solution is neutralized and the pH adjusted to 8.5–9. The lead is then extracted with a chloroform solution of dithizone. The extracted lead dithizonate is decomposed with 1 per cent nitric acid which transfers the lead to the aqueous phase. The lead is finally determined electrolytically as described in Chapter 21, *Methods of Analyses*.

Samples were sent to three manufacturers who had expressed willing-

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 51 (1949).

¹ *This Journal*, 30, 552 (1947).

ness to collaborate and to three chemists in the Food and Drug Administration. Two of the three firms have not yet reported results, because of lack of the necessary electrolytic equipment. However, it is hoped that they will be able to report in the near future.

TABLE 1.—*Collaborative results*

COLLABORATOR	RECOVERY MICROGRAMS	DEVIATION FROM MEAN
Associate Referee	118	+1
	119	+2
2	116	-1
	117	0
3	118	+1
	119	+2
4	119	+2
	121	+4
5	110	-7
	109	-8
Mean	117	
Average Deviation		2.6
Standard Deviation		3.7

The results of the several collaborators (Table 1) give an average deviation from the mean of 2.6 micrograms and a standard deviation of 3.7 micrograms. The highest result is 121 micrograms; the lowest is 109 micrograms.

One collaborator commented that he "found this method better than the double extraction colorimetric method for several reasons, namely, (1) shorter time required; (2) less chloroform required; and (3) fewer pitfalls due to the final electrolytic method of determination."

The author wishes to thank P. A. Clifford, L. S. Harrow, N. Gordon, and H. Holtzman for collaborative results reported.

RECOMMENDATION*

It is recommended that the proposed method be adopted as official, first action, for the determination of lead in all straight coal-tar colors listed as certifiable under the regulations promulgated in accordance with the Federal Food, Drug, and Cosmetic Act, except those containing calcium, barium, or strontium.†

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 51 (1949).

† Details of the method are given in *This Journal*, 32, 88 (1949).

REPORT ON NON-VOLATILE, UNSULFONATED, AMINE INTERMEDIATES IN COAL-TAR COLORS

By LEE S. HARROW (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

3-NITRO-P-TOLUIDINE

D&C Red No. 35 (also known as Toluidine Red, C. I. 69) and D&C Red No. 38 (also known as Toluidine Maroon) are colors certifiable by the Food and Drug Administration for coloring drugs and cosmetics (1). The maximum amount of free intermediate, 3-nitro-p-toluidine, permitted in a certified batch of these colors is 0.2 per cent.

In a paper presented at the annual meeting of the A.O.A.C. (October 1947) a method was proposed for the separation and quantitative determination of p-nitroaniline in D&C Black No. 1 (2). The method described here is a modification of that method which makes it applicable to the separation and quantitative determination of 3-nitro-p-toluidine in D&C Red No. 35 or D&C Red No. 38. The 3-nitro-p-toluidine is extracted from the colors with petroleum benzin and titrated with titanium trichloride (3).

METHOD

Place a 10 g sample of D&C Red No. 35 or D&C Red No. 38 in a Soxhlet extraction thimble of suitable size and extract with petroleum benzin for 6-8 hours. Transfer extract to 500 ml wide-mouth Erlenmeyer flask. Rinse the Soxhlet flask with two 10 ml portions of petroleum benzin and add these to main extract. Add 50 ml of water to combined extracts and heat on steam bath until all petroleum benzin is removed, using a gentle air current to hasten the process. Remove flask from steam bath and add ca 15 g of sodium tartrate, heat soln to boiling, and titrate with standard 0.1 N TiCl_3 soln under a stream of CO_2 to disappearance of yellow color. The end point can be more readily detected when 1 ml of a standard soln of FD&C Green No. 2 (Light Green SF Yellowish) is added near end of the titration to serve as an indicator.

RESULTS

Samples of D&C Red No. 35 and D&C Red No. 38 were extracted with petroleum ether until intermediate free; known quantities of 3-nitro-p-toluidine were added to 10 gram portions of these intermediate free colors. Satisfactory recoveries of the added intermediates were obtained when these samples were analyzed by the proposed method. Results of these analyses are shown in Tables 1 and 2.

Further investigations are being conducted to determine the applicability of the method to 2-4-dinitroaniline and 2-nitro-p-anisidine in colors in which they may be encountered.

P-NITROANILINE

A collaborative study of the method proposed for the determination of p-nitroaniline in D&C Black No. 1 (2) has been made.

TABLE 1.—*Recovery of 3-nitro-p-toluidine from D&C Red No. 35*

3-NITRO-P-TOLUIDINE ADDED	3-NITRO-P-TOLUIDINE RECOVERED	RECOVERY
<i>gram</i>	<i>gram</i>	<i>per cent</i>
0.00	0.00	0.0
0.0205	0.0195	94.8
0.0224	0.0214	95.6
0.0195	0.0190	97.2
0.0400	0.0391	97.7
0.0395	0.0383	96.8
0.0417	0.0409	98.0
0.0592	0.0584	98.8
0.0629	0.0631	100.3
0.0603	0.0598	99.3
Average Recovery		97.6

TABLE 2.—*Recovery of 3-nitro-p-toluidine from D&C Red No. 38*

3-NITRO-P-TOLUIDINE ADDED	3-NITRO-P-TOLUIDINE RECOVERED	RECOVERY
<i>grams</i>	<i>grams</i>	<i>per cent</i>
0.00	0.00	0.0
0.0224	0.0224	100.0
0.0196	0.0193	98.5
0.0230	0.0224	97.3
0.0408	0.0398	97.6
0.0399	0.0391	98.0
0.0404	0.0399	98.7
0.0590	0.0591	100.2
0.0603	0.0598	99.2
0.0613	0.0603	98.4
Average Recovery		98.7

A composite sample of D&C Black No. 1 was prepared and sent to the following collaborators for study:

Ansbacher-Siegle Corporation—H. Holtzman reporting.

National Aniline Division, Allied Chemical & Dye Corp.—A. T. Schramm, reporting.

Calco Chemical Division, American Cyanamid Company—William Seaman, E. Z. Montgomery, and W. H. McComas, Jr. reporting.

Thomasset Colors, Inc.—A. Cohen reporting.

Wm. J. Stange Company—W. H. Kretlow reporting.

H. Kohnstamm and Company, Inc.—I. Hanig reporting.

The results submitted are given in Table 3.

TABLE 3.—*p*-nitroaniline in D&C Black No. 1

COLLABORATOR	D-NITROANILINE	COLLABORATOR	D-NITROANILINE
	<i>per cent</i>		<i>per cent</i>
1	0.15	5	0.12
	0.15		0.12
			0.13
2	0.19	6	0.12
	0.19		0.09
3	0.13	Average.....	0.14
	0.13		
4	0.17		

RECOMMENDATIONS*

It is recommended—

(1) That collaborative work be done on 3-nitro-*p*-toluidine in D&C Red No. 35 and D&C Red No. 38.

(2) That additional collaborative work be done on *p*-nitroaniline in D&C Black No. 1.

(3) That the topic Non-Volatile, Unsulfonated, Amine Intermediates in Coal-Tar Colors be continued.

REFERENCES

- (1) S.R.A., F.D.C. 3, U. S. Food and Drug Administration.
- (2) HARROW, L. S., *This Journal*, 31, 594 (1948).
- (3) KNECHT and HIBBERT, "New Reduction Methods in Volumetric Analysis," Longmans (1918).

REPORT ON SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS

IDENTIFICATION OF FLUORESC EIN COLORS

By RACHEL N. SCLAR (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

There are fifteen individual colors of the fluorescein type (excluding the salts of the listed color acids) on the list of certifiable colors (1). In the qualitative analysis of food, drug, and cosmetic products for coal-tar dyes, it is usually an easy task to separate the fluorescein-type dyes from

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 51 (1949).

the other dyes present and establish their presence. The positive identification of the particular fluorescein dye, or dyes, present, however, is a more difficult task.

This report covers a preliminary study of the spectrophotometric char-

TABLE 1.—*Fluorescein colors, in order of increasing wave length of absorption maximum*

COLOR	OTHER NAME	WAVE LENGTH OF ABSORPTION MAXIMUM	REMARKS
D&C Yellow No. 7	Fluorescein	490	Strong fluorescence
D&C Orange No. 5	Dibromofluorescein	504	
*D&C Orange No. 14	Tribromodicarboxy-fluorescein	504	
*Ext. D&C Orange No. 2	Dinitrofluorescein	504	Strong fluorescence
*D&C Orange No. 8	Dichlorofluorescein	504	
*D&C Orange No. 10	Diiodofluorescein	508	
*D&C Red No. 24	Tetrachlorofluorescein	509	Strong fluorescence marked shoulder at 475 m μ .
D&C Red No. 21	Tetrabromofluorescein	517	
*D&C Red No. 29	Pentabromodicarboxy-fluorescein	517	
*D&C Orange No. 16	Diiododibromofluorescein	521	Very slight (almost no) shoulder.
*FD&C Red No. 3	{ Erythro.ine Na salt of tetraiodo-fluorescein	527	
*Ext. D&C Red No. 3	Violamine R	530	
*D&C Red No. 27	Tetrachlorotetrabromo-fluorescein	539	Marked shoulder at 505 m μ .
*Ext. D&C Red No. 4	Dichlorotetraiodo-fluorescein	546	
D&C Red No. 19	Rhodamine B	553	

* Commercial samples.
Color acids run in dilute NH₄OH.
Na salts run in plain H₂O.

acteristics of the certifiable fluorescein dyes. The work reported here gives a method for the spectrophotometric identification of any of the fifteen certifiable fluorescein colors, provided that only one color of this type is present.

The absorption spectra of the solutions of various fluorescein colors are, as would be expected, very similar in shape. The chief difference in the respective curves of colors of this type is the position of the absorption maximum. The wave length of the maximum extinction for dilute am-

moniacal solutions of the fifteen certifiable fluorescein colors is shown in Table 1.

Table 1 shows that in many curves the wave length of maximum absorption alone is sufficiently distinctive to identify the color. In several other curves where two dyes have very nearly the same wave length of maximum absorption, qualitative tests for the halogens and nitrogen will provide more definite identification.

It will be noted, however, that there are two pairs of colors, D&C Oranges No. 5 and 14, and D&C Reds No. 21 and 29, the members of which cannot be distinguished in this manner. Attempts to differentiate these dyes on the basis of differences in the location of the absorption peak in various solvents were unsuccessful. In an attempt to determine the isosbestic points of these colors, however, it was noted that there are observable differences in the spectrophotometric curves of these colors at certain *pH* values. Further work showed that these differences could be used to identify the colors.

EXPERIMENTAL

All optical measurements were made with a General Electric recording spectrophotometer equipped with slit adjustments for an 8-millimicron wave length band. To minimize the effect of the fluorescence of the dye, the cells containing the solutions used in this work were placed at the forward end of the transmission compartment, approximately five inches from the integrating sphere. Calculations indicate that under these conditions less than one per cent of the fluorescent light emitted by the sample should reach the integrating sphere.

The solutions used to obtain the data shown in Table 1 contained 0.5 ml of concd. ammonium hydroxide per 100 ml. For most of these colors the same curve is obtained at any *pH* above 6.0.

Solutions for the spectrophotometric determinations in the study of the effect of *pH* on the curves were prepared by pipeting 25 ml of the alcoholic dye solution into a 50 ml volumetric flask, and adding the required amount of buffer. The resulting solutions were made to volume with water. The buffer mixtures were prepared as directed by Clark and Lubs (2), except that the concentrations of the stock solutions were two to five times that specified to allow for subsequent dilution. (The *pH* values quoted are those the buffer mixtures would give in water alone. It is realized that these values may not represent the actual *pH* of the alcoholic solutions.)

DISCUSSION

Table 2 gives the data obtained on solutions of D&C Yellow No. 7, D&C Oranges No. 5 and 14, and D&C Reds No. 21 and 29, at various *pH* values. Typical sets of curves for each of these dyes are shown in Figures 1-5.

The data for fluorescein shows that the wave length of maximum ab-

TABLE 2.—Wave length of absorption maximum and extinction at absorption maximum

pH	FIG. 1 D&C YELLOW #7		FIG. 2 D&C ORANGE #5		FIG. 3 D&C ORANGE #14		FIG. 4 D&C RED #21		FIG. 5 D&C RED #29	
	WAVE LENGTH	EXTINC- TION	WAVE LENGTH	EXTINC- TION	WAVE LENGTH	EXTINC- TION	WAVE LENGTH	EXTINC- TION	WAVE LENGTH	EXTINC- TION
	mμ	% max	mμ	% max	mμ	% max	mμ	% max	mμ	% max
1.4							524	5.3	528	7.4
2.0							529	13.8	533	17.5
2.2							529	29.7	533	35.4
2.4							530	47.1	533	50.1
2.6							530	61.8	533	61.7
3.0			529	6.4	472	3.9	530	82.8	533	76.0
3.4			528	7.8	470-500	4.9				
3.8	454	1.6	525	10.0	516	7.8	529	98.8	531	88.8
4.0	454	2.9	517	19.0	514	21.3	527.5	97.1	528	91.3
4.2	454	5.8								
4.4	454 & 478	9.5	511	59.8	513	60.2				
4.8	481	20.3	510	83.6	512	77.5	523	98.3	525	98.5
5.2	489	35.9	510	93.4	511	87.3				
5.6	494	59.0								
6.0	495	78.8					522	100.0	524	100.0
8.0	496	100.0					522	100.0	524	100.0
8.4	496	100.0	509	100.0	510	100.0				

sorption shifts to shorter wave lengths as the pH is lowered. The extinction per milligram in the visible region decreases rapidly as the pH is lowered and is very low at any pH below 4. At all pH values less than 5, the curve shows a double peak.

The absorption curves for solutions of D&C Oranges No. 5 and 14 show that below pH 4, there are distinct differences in the location of the absorption maxima and the shape of the curves. For D&C Orange No. 5, the absorption maximum moves toward the longer wave lengths as the pH is lowered, but the shape of the curve remains the same. For D&C Orange No. 14, however, the maximum present at pH 4 flattens out as the pH is decreased, and a new absorption maximum appears at 472 mμ. The wave length of maximum extinction at pH 3.8 for D&C Orange No. 5 is 525 mμ and for D&C Orange No. 14, 516 mμ. There is also a greater proportional increase in the extinction at pH 6 over that at pH 3.4 for D&C Orange No. 14 than for D&C Orange No. 5. The ratio of the extinction at pH 6 to that of pH 3.4 is 12.9 for D&C Orange No. 5 and 23.4 for D&C Orange No. 14.

Since solutions of D&C Yellow No. 7 below a pH of 4 show very little absorption at 510 mμ, moderate contamination of D&C Orange No. 5 with D&C Yellow No. 7 has little effect on the location of the absorption peak or extinction values at the peak for solutions of D&C Orange No. 5 at the low pH values. The ratio $E_{pH\ 6.0}/E_{pH\ 3.4}$ is, however, increased and may be used to estimate the amount of D&C Yellow No. 7 present in samples of D&C Orange No. 5.

The differences between D&C Reds No. 21 and 29 are not as great as

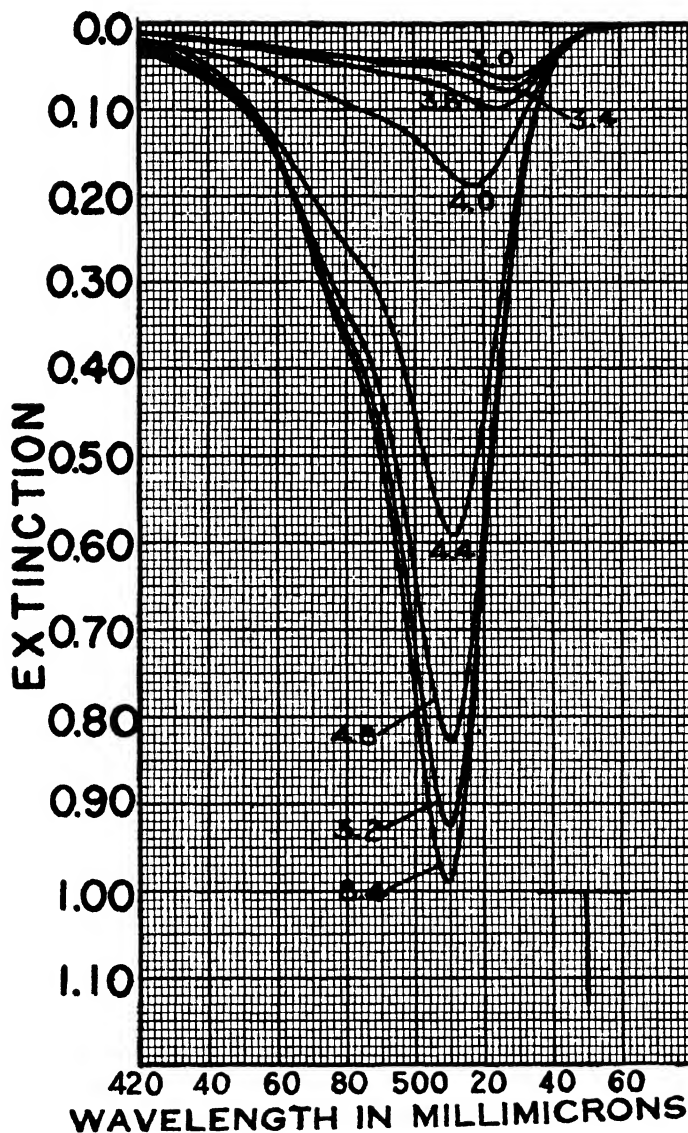


FIG. 2.—Extinction of solutions of D&C Orange No. 5, 6.0 mg./liter, at various pH values. Solvent—50% alcohol. Cells—1 cm.

those between D&C Oranges Nos. 5 and 14. Examination of the data, however, does show distinctive differences in the behavior of the two colors. For both colors, the absorption peak shifts steadily toward shorter wave lengths as the pH is raised. For D&C Red No. 29, the maximum extinction value increases continuously as the pH is raised until it reaches a limiting value at about pH 6. For D&C Red No. 21, however, the maximum

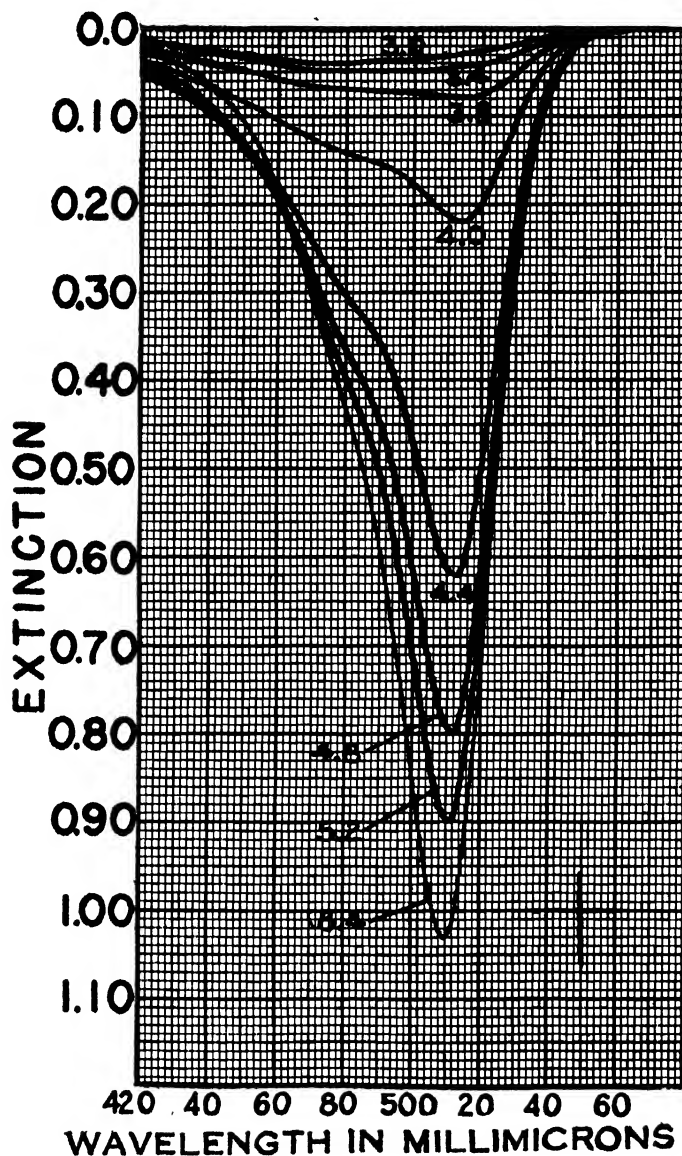


FIG. 3.—Extinction of solutions of D&C Orange No. 14, 7.0 mg./liter, at various pH values. Solvent—50% alcohol. Cells—1 cm.

extinction value increases until the pH is 3.8, is less at pH 4.0 and 4.4 than at 3.8, and finally increases to a limiting value at about pH 6.0.

The ratio $E_{pH\ 3.8}/E_{pH\ 2.0}$ is also useful as a supplementary means of identification. This ratio is 7.2 for D&C Red No. 21, and 5.2 for D&C Red No. 29.

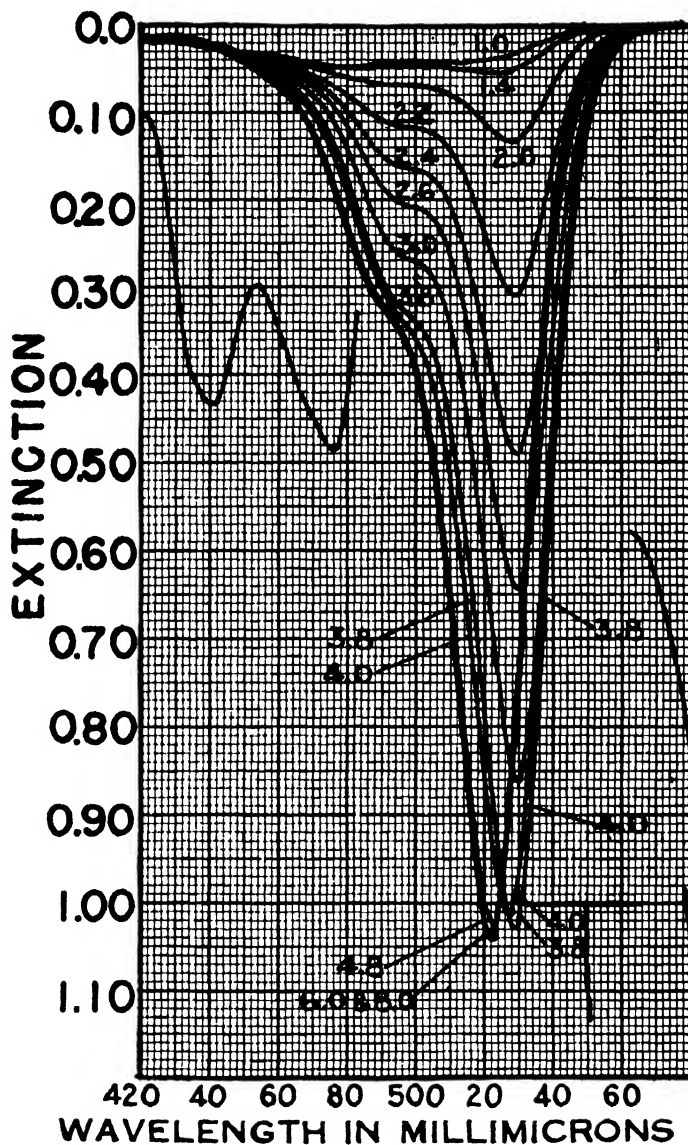


FIG. 4.—Extinction of solutions of D&C Red No. 21, 6.4 mg./liter, at various pH values. Solvent—50% alcohol. Cells—1 cm.

Curves for solutions of D&C Red No. 21 containing 2.0–22.0 per cent of added D&C Orange No. 5, at pH values of 2.0, 3.8, 4.0, and 4.4, were drawn. The distinctive decrease in the extinction for D&C Red No. 21 at pH 4.0 was not eliminated, although it became less apparent at the highest percentage of D&C Orange No. 5. The extinction value for the absorption peak at pH 4.4 increased very rapidly as the percentage of

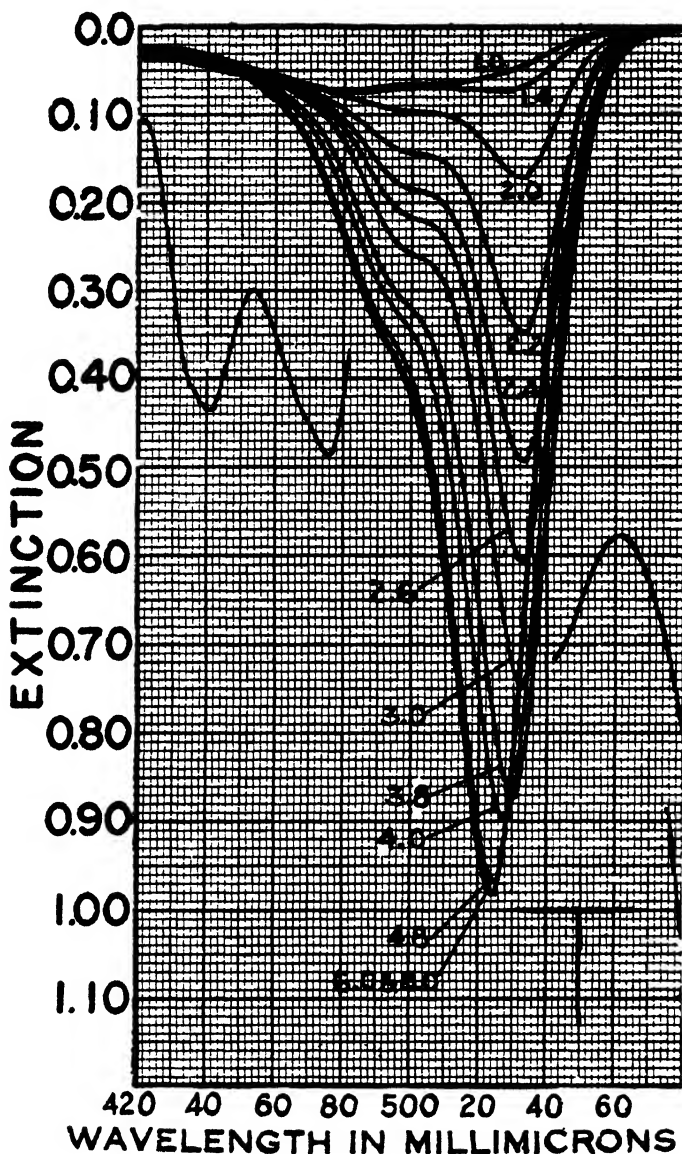


FIG. 5.—Extinction of solutions of D&C Red No. 29, 7.5 mg./liter, at various pH values. Solvent—50% alcohol. Cells—1 cm.

orange dye was raised and should provide an estimate of the amount of D&C Orange No. 5 present in the D&C Red No. 21.

APPLICATIONS

It should be noted that it is not necessary to obtain the curves at all the pH values listed in Table 2 to identify the dyes. Curves for solutions at

pH 3.0, 3.4, 3.8, and 6.0 are sufficient to identify D&C Oranges No. 5 and 14, while curves at pH 2.0, 3.8, 4.0, and 4.4 are sufficient to identify D&C Reds No. 21 and 29.

Samples of lipsticks were prepared containing the following percentages of commercial dyes:

Fluorescein dye	2.0
D&C Red No. 17.	0.5
D&C Red No. 8.	10.0

The samples were examined as unknowns and the fluorescein dye present identified without difficulty.

The data given in this report have been used to show that a sample of D&C Orange No. 5, which had a low bromine content, contained a considerable amount of unbrominated fluorescein.

It is planned to extend the study to determine the effect of pH on the absorption curves of solutions of the other certifiable fluorescein dyes and the non-certifiable fluorescein colors.

SUMMARY

Data on the wave length of maximum absorption for dilute ammoniacal solutions of the fifteen certifiable colors are given. These data will permit identification of most of these colors, provided only one color of this type is present. In other cases, qualitative analysis for halogens or nitrogen will be needed to complete the identification.

D&C Oranges No. 5 and 14 (and D&C Reds No. 21 and 29) cannot be differentiated on this basis. It is shown, however, that these colors can be identified from the spectrophotometric curves of solutions of the colors in fifty per cent alcohol solution at several pH levels.

Data on the location and magnitude of the absorption peak of solutions of D&C Yellow No. 7, D&C Oranges No. 5 and 14, and D&C Reds No. 21 and 29 at various pH values are presented and discussed.

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- (1) S.R.A., F.D.C. 3—U. S. Food and Drug Administration, Washington, D. C.
- (2) LANGE, N. A., *Handbook of Chemistry*, 6th Ed., pp. 1102-1103, Handbook Publishers, Inc., Sandusky, Ohio.

REPORT ON SPECTROPHOTOMETRIC ANALYSIS OF COAL-TAR COLORS

D&C GREEN NO. 6

By RACHEL N. SCLAR (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

This report on the spectrophotometric determination of D&C Green No. 6 is a continuation of the program for the spectrophotometric analysis

of oil-soluble dyes certifiable under the Coal-Tar Color Regulations (1). Previous reports gave spectrophotometric data for Ext. D&C Yellow No. 5 (2), D&C Red No. 35 (3), D&C Red No. 36 (3), Ext. D&C Orange No. 1 (4), and for D&C Violet No. 2 (5).

As before, conformity to Beer's law, location of the absorption peaks, and the extinction ratio at suitable wave lengths were determined for solutions of the dye.

EXPERIMENTAL

All the spectrophotometric data were determined with a General Electric recording spectrophotometer equipped with an automatic slit adjustment for an 8-millimicron wave length band.

Preparation of D&C Green No. 6 (Quinizarin Green)

One part of leucoquinizarin (obtained by reducing quinizarin—m.p. 195°C., with stannous chloride) was condensed with about eight parts of p-toluidine (m.p. 44°C.) in the presence of anhydrous boric acid and chalk. The resulting leuco dye was allowed to reoxidize in air to form quinizarin green. These processes were combined in one operation (6). The dye was boiled with dilute hydrochloric acid, washed with water until neutral, then boiled with dilute sodium hydroxide, washed, and dried. The dye was recrystallized three times from glacial acetic acid. Melting point (on Fisher block), 219–219.5°C. Recrystallization of this material from chloroform did not change the melting point. Literature, m.p. 218°C. (7).

The dye appears to pass through a transition stage at about 213–214°C. At that temperature it liquefies, but resolidifies upon further heating and finally melts sharply at 219–219.5°C. The pure dye, when heated in a crucible on a hot plate until completely liquid, cooled, and allowed to crystallize, melts at 219°C without preliminary liquefaction. Spectrophotometric examination of a solution of the standard dyestuff prior and subsequent to the heating treatment showed no change in spectrophotometric characteristics.

The purified material adsorbed on a column of activated alumina from petroleum ether and developed with benzene appeared homogeneous. A commercial preparation of D&C Green No. 6 when chromatographed in the same manner gave two bands (mauve and green). The mauve fraction was not definitely identified, but appeared to be unreacted quinizarin.

A portion of the commercial sample of D&C Green No. 6 was washed with hot dilute sodium hydroxide, hot water, hot dilute hydrochloric acid, dried and recrystallized once from glacial acetic acid. This material melted at 214°C. Two recrystallizations from chloroform raised the melting point to 218°C. Recrystallization from benzene brought the melting point to 218.5°C. The purified material was spectrophotometrically identical with the laboratory preparation and gave a homogeneous chromatogram. The sample prepared in this laboratory was, therefore, considered sufficiently pure to serve as a standard for D&C Green No. 6.

Spectrophotometric Data

The dye, weighed on a semimicro balance sensitive to 0.02 mg, was dissolved in about 50 ml of chloroform in a 100 ml volumetric flask. The solution was made to volume with chloroform, aliquot portions were diluted to a definite concentration, and the spectrophotometric curve determined. (All solutions were made to volume at the temperature of the room in which the optical measurements were made. U.S.P. chloroform was used throughout the experiment.)

In subsequent experiments identical spectrophotometric curves were obtained when gentle warming on a water bath was used to facilitate solution of the color in the solvent.

A typical set of data is shown in Table 1.

TABLE 1.—*Extinction values of solutions of D&C Green No. 6 in U.S.P. chloroform*

Typical data

CURVE NO. (CHART 1)	CONCENTRATION	EXTINCTION			$E_{630\text{ m}\mu}$	$E_{660\text{ m}\mu}$
		630 m μ	645 m μ	660 m μ	CONCENTRATION	$E_{630\text{ m}\mu}$
1	mg./liter 7.70	.288	.304	.272	.0395	1.059
2	15.41	.572	.608	.544	.0395	1.051
3	30.81	1.156	1.220	1.092	.0396	1.059
Average0395	1.06

Typical extinction curves for chloroform solutions of D&C Green No. 6 are shown in Figure 1. The curves show a minor absorption peak at approximately 412, and a characteristic double peak in the red area. The major absorption peak is at $645 \pm 2 \text{ m}\mu$. (All wave lengths were corrected to $\pm 2 \text{ m}\mu$ with the aid of didymium glasses calibrated by the National Bureau of Standards; see footnote to Figures 1 and 2.)

The average extinction per milligram per liter for D&C Green No. 6, calculated from the results of 30 determinations (at various concentrations) made from ten portions of the dye is 0.0395. The average deviation from the mean for these determinations was 0.2 per cent, and the maximum deviation 0.5 per cent.

Point readings of extinction values were taken at arbitrarily chosen wave lengths, 630 m μ and 660 m μ on opposite sides of the major absorption peak. The ratio of extinction values ($E_{630 \text{ m}\mu}/E_{660 \text{ m}\mu}$) at these wave lengths was $1.06 \pm .01$ (see Table 1).

A chloroform solution of the dye, stored for three days in the dark, gave spectrophotometric data identical with that of the freshly prepared solutions.

Application to Commercial Samples

Three samples of certified D&C Green No. 6 (straight colors) were analyzed spectrophotometrically. Weighed samples were dissolved in

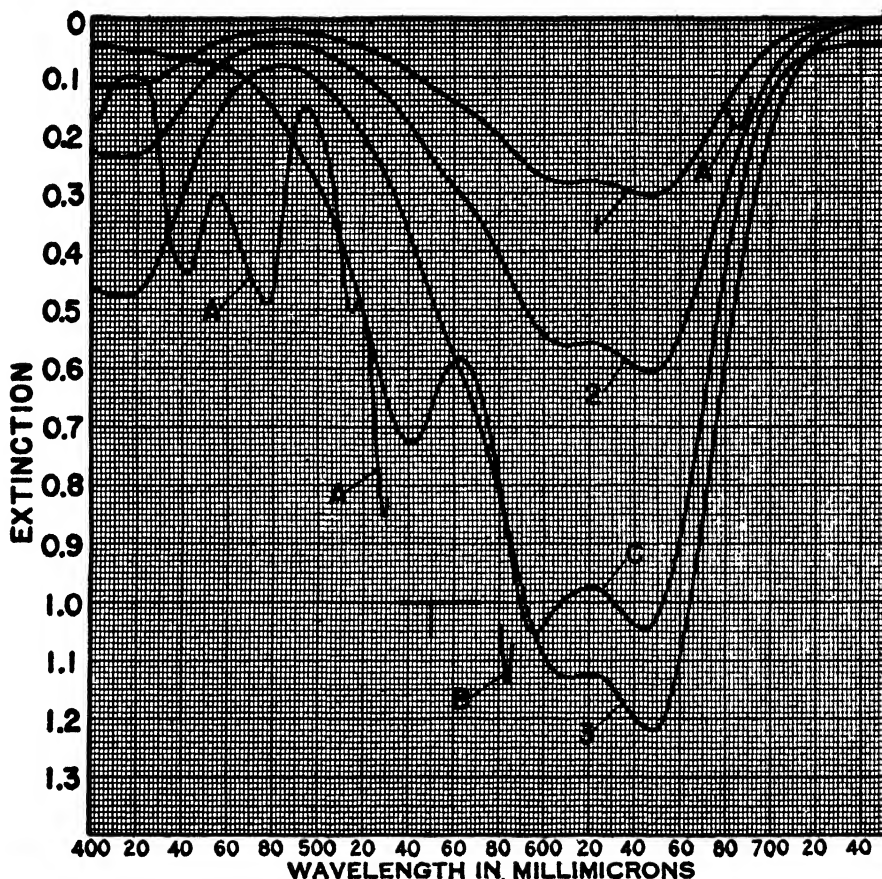


FIG. 1.—Solutions of D&C Green No. 6 in U.S.P. chloroform.

Curve 1— 7.70 mg./liter

Curve 2—15.41 mg./liter

Curve 3—30.81 mg./liter

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$).

B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 $m\mu$).

C = Signal Lunar White Glass-H-6946236.

U.S.P. chloroform by warming on the steam bath. The solutions were transferred to 100 ml flasks, cooled, and made to volume at room temperature. Extinction measurements were made on appropriately diluted aliquots. The curves are shown in Figure 2, and the data in Table 2.

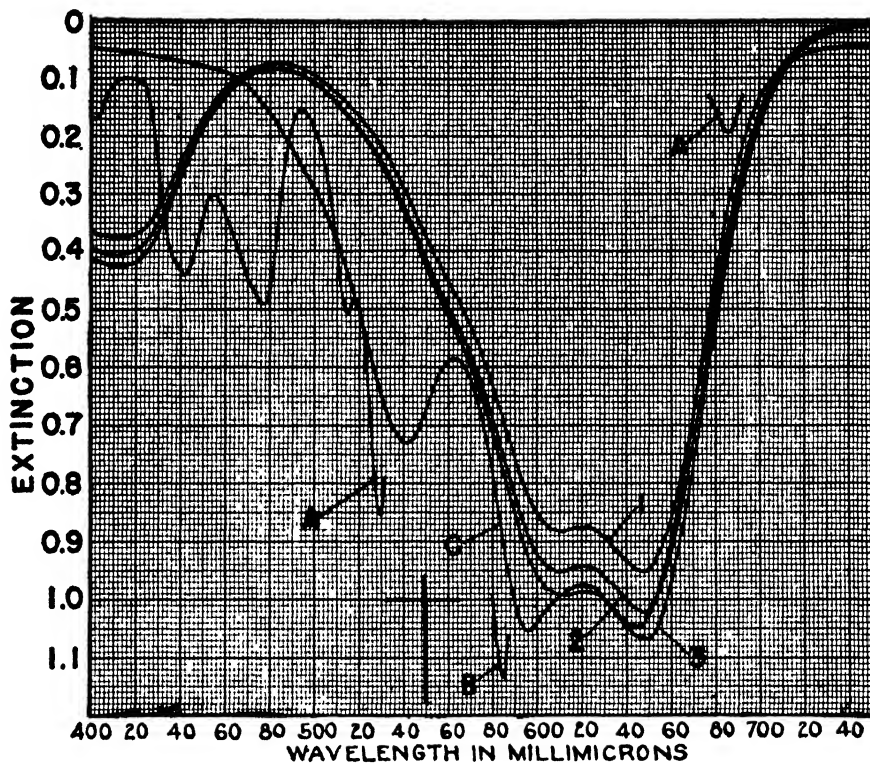


FIG. 2.—Certified samples of D&C Green No. 6.

Curve 1—24.69 mg./liter

Curve 2—26.74 mg./liter

Curve 3—27.60 mg./liter

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$).

B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 $m\mu$).

C = Signal Lunar White Glass-II-6946236.

TABLE 2.—Analysis of certified samples of D&C Green No. 6

Straight color

SAMPLE NO.	M.P.	CONCENTRATION OF SAMPLE	$E_{550m\mu}$	DYE* SPECTROPHOTOMETRICALLY	DYE FROM NITROGEN CONTENT
		mg./liter		per cent	
1	213-214	24.69	0.952	97.6	97.2
2	216.5-217.5	26.74	1.020	96.6	98.0
3	218	27.60	1.067	97.9	98.3

* The dye content was calculated by using 0.0395 (Table 1) as the extinction value for 1 mg./liter of pure D&C Green No. 6.

DISCUSSION

The ratios of extinction to concentration in Table 1 show that at 645 $m\mu$ chloroform solutions of D&C Green No. 6 containing 7 to 31 mg of color per liter obey Beer's law. The "pure dye" content of a sample of this color can therefore be determined from the ratio of the extinction of a chloroform solution of the sample to that of a standard solution of the pure dye at 645 $m\mu$.

SUMMARY

Spectrophotometric data for chloroform solutions of pure D&C Green No. 6 are presented. The dye is shown to follow Beer's law. The major absorption peak is at $645 \pm 2 m\mu$. The extinction per milligram per liter at 645 is $.0395 \pm .0002$. The extinction ratio $E_{630 m\mu}/E_{660 m\mu} = 1.06 \pm .01$. Chloroform solutions of the dye are stable for at least three days if stored in the dark.

Application is made of these data to the determination of the pure dye content of commercial samples of the color. Typical results are given.

REFERENCES

- (1) S.R.A., F.D.C. 3, U. S. Food and Drug Administration.
- (2) CLARK, G. R., and NEWBURGER, S. H., "Spectrophotometric Analysis of Coal-Tar Colors I, Ext. D&C Yellow No. 5," *This Journal*, **27**, 576 (1944).
- (3) SCLAR, RACHEL, N., "Report on Spectrophotometric Testing of Coal-Tar Colors: D&C Red Nos. 35 and 36," *This Journal*, **30**, 522 (1947).
- (4) SCLAR, RACHEL, N., "Report on Spectrophotometric Testing of Coal-Tar Colors: Ext. D&C Orange No. 1," *This Journal*, **31**, 598 (1948).
- (5) DOLINSKY, MEYER, "Spectrophotometric Testing of D&C Violet No. 2," *This Journal*, **31**, 674 (1948).
- (6) FIERZ-DAVID, H. E., *Grundlegende Operationen der Farbenchemie*, 5th Ed., p. 300 (1943), Edwards Brothers, Inc., Ann Arbor, Michigan.
- (7) FRIEDLANDER, SCHICK, *Chemis. Zentr.*, **75**, Band 2, 339 (1904).

REPORT ON SUBSIDIARY DYES IN COAL-TAR COLORS

SPECTROPHOTOMETRIC DETERMINATION OF D&C ORANGE NO. 4 IN SAMPLES OF FD&C ORANGE NO. 1

By MEYER DOLINSKY (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The Coal-Tar Color Regulations permit a maximum of 5.0 per cent D&C Orange No. 4 in certifiable batches of FD&C Orange No. 1 (1). The tentative A.O.A.C. method (2) for the determination of D&C Orange No. 4 in samples of FD&C Orange No. 1 is not very satisfactory for routine analytical work, since it requires numerous extractions and takes $1\frac{1}{2}$ to 2 hours for each analysis.

It was found that the number of extractions and the time required could

be cut in half by taking a 50 to 100 mg sample and determining the extracted D&C Orange No. 4 spectrophotometrically. However, even this modified procedure was felt to be too slow. Furthermore, results obtained by the extraction procedure are usually low because of mechanical losses and incomplete separation of D&C Orange No. 4 from the FD&C Orange No. 1. Recoveries of D&C Orange No. 4 obtained when extracted alone and in the presence of FD&C Orange No. 1 are shown in Table 1.

TABLE 1.—*Recoveries of D&C Orange No. 4 by extraction procedure*

FD&C ORANGE NO. 1	D&C ORANGE NO. 4	D&C ORANGE NO. 4 RECOVERED
<i>mg</i>	<i>mg</i>	<i>per cent</i>
98	12 (modified procedure)	93
0	2 " "	91
0	50 " "	83
977	63 (A.O.A.C. procedure)	65

Because of the disadvantages of the extraction procedure it was felt that a more rapid and precise method would be desirable. In this connection it was decided to investigate a spectrophotometric method for the determination.

The absorption curves of neutral solutions of FD&C Orange No. 1 and D&C Orange No. 4 are quite similar, but there is a marked difference

TABLE 2.—*Spectrophotometric analysis of solutions containing purified D&C Orange No. 4 and FD&C Orange No. 1*

FD&C ORANGE NO. 1		D&C ORANGE NO. 4		RECOVERY OF D&C ORANGE NO. 4
ADDED	FOUND	ADDED	FOUND	
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
90.9	91.4	9.1	8.6	95
90.9	90.8	9.1	9.2	101
90.9	91.6	9.1	8.4	92
95.2	94.7	4.8	5.3	110
95.2	95.4	4.8	4.6	96
95.2	96.0	4.8	4.0	83
97.5	97.6	2.5	2.4	96
97.5	97.3	2.5	2.7	108
98.0	98.5	2.0	1.5	75
99.0	98.8	1.0	1.2	120
99.0	98.8	1.0	1.2	120
Average Recovery				100
Average Error				11
Maximum Error				25

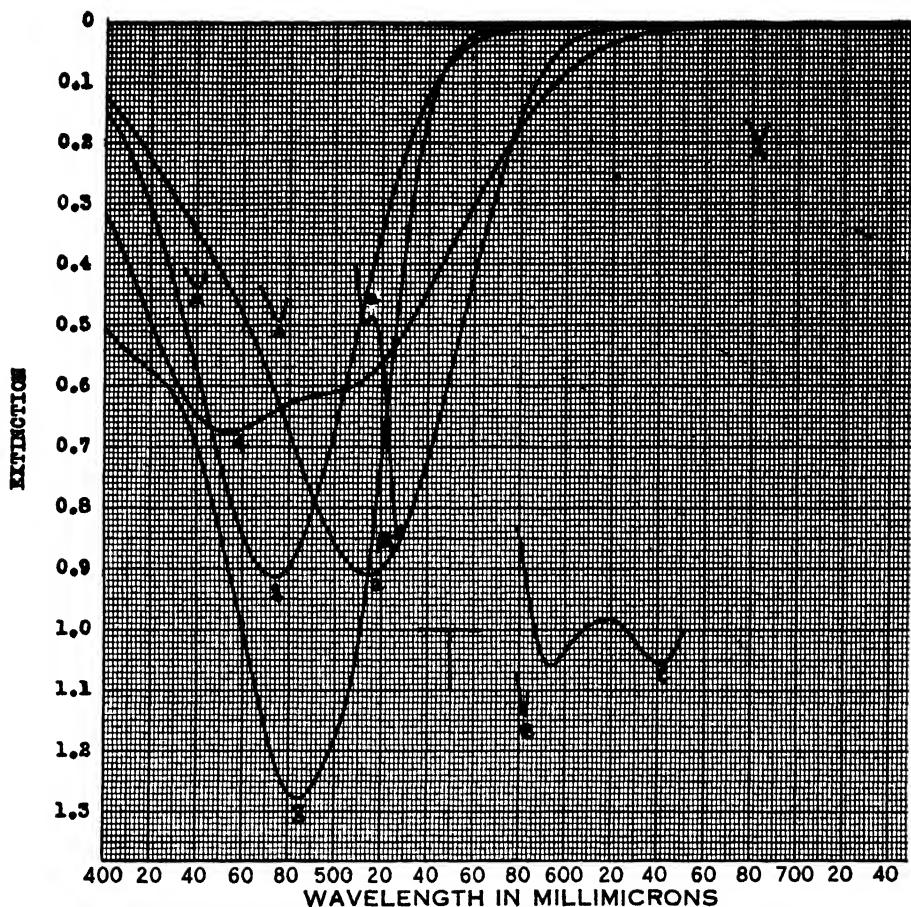


Fig. 1.—Absorption curves of FD&C Orange No. 1 and D&C Orange No. 4 in neutral and in 0.1 *N* NaOH solution.

Curve 1—FD&C Orange No. 1—Neutral —10 mg./liter.

Curve 2—FD&C Orange No. 1—In 0.1 *N* NaOH—10 mg./liter.

Curve 3—D&C Orange No. 4 —Neutral —20 mg./liter.

Curve 4—D&C Orange No. 4 —In 0.1 *N* NaOH—20 mg./liter.

Cells—1 cm.

A=Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$).

B=Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 $m\mu$).

C=Signal Lunar White Glass-II-6946236.

when the colors are dissolved in 0.1 *N* sodium hydroxide solution (Fig. 1). Each of the dyes follows Beer's law in 0.1 *N* sodium hydroxide solution. It is possible, therefore, to calculate the amount of each dye present in a mixture of the two from the absorption spectra of solutions of the mixture and the individual components by the use of simultaneous equations.

EXPERIMENTAL

Optical measurements were made with a General Electric Recording spectrophotometer and with a Beckman Quartz spectrophotometer.

The purified D&C Orange No. 4 used as a standard in these studies was obtained by twice recrystallizing a certified sample from alcohol. The purified FD&C Orange No. 1 was obtained by twice recrystallizing a certified sample from alcohol-water. Both colors were dried at 135°C. prior to use.

A suitable aliquot of a 0.02% solution of purified D&C Orange No. 4 was added to a definite amount of a 0.02% solution of FD&C Orange No. 1 and the mixture diluted with water and sufficient 0.4 *N* sodium hydroxide to give the solutions for spectrophotometric analysis. The densities of the mixture and the standards at 455 m μ and 515 m μ were determined and the percentage of each dye in the mixture calculated by the method of simultaneous equations. Results are shown in Table 2.

Two certified samples of FD&C Orange No. 1 were analyzed for D&C Orange No. 4 by both the extraction and spectrophotometric procedures. Results are shown in Table 3.

TABLE 3.—*Spectrophotometric determination of D&C Orange No. 4 in commercial samples of FD&C Orange No. 1*

SAMPLE	D&C ORANGE NO. 4 SPECTROPHOTOMETRICALLY	D&C ORANGE NO. 4 BY EXTRACTION
No. 1	<i>per cent</i>	<i>per cent</i>
	3.1 (Beckman)	2.1 (Average of four determinations) (A.O.A.C. and Modified Procedure)
	3.0 (G.E.)	
	2.9 (G.E.)	
	2.4 (G.E.)	
No. 2	4.0 (Beckman)	2.9 (Modified Procedure)
	3.6 (G.E.)	

SUMMARY

A rapid spectrophotometric method for the determination of D&C Orange No. 4 in samples of FD&C Orange No. 1 is presented.

The method is shown to be applicable to mixtures containing 1.0 to 9.1 per cent of pure D&C Orange No. 4 and 99.0 to 90.9 per cent of pure FD&C Orange No. 1, with an average error of 11.1 per cent. Values obtained for D&C Orange No. 4 in two commercial samples of FD&C Orange No. 1 by the spectrophotometric method were higher than those obtained by the A.O.A.C. extraction procedure.

It is recommended* that the method for D&C Orange No. 4 in FD&C Orange No. 1 be submitted to collaborative study, and that the topic be continued.

* For report of Subcommittee B and action of the Association, see *This Journal*, 32, 51 (1949).

REFERENCES

- (1) SRA. F.D.C. 3—Food and Drug Administration.
 - (2) *Methods of Analysis*, A.O.A.C., 6th Ed. (1945).
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REPORT ON PURE DYE IN LAKES AND PIGMENTS

By KENNETH A. FREEMAN (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The tentative procedure (1) for the determination of pure dye in lakes of D&C Red No. 8 and D&C Red No. 31 has been shown to be applicable to D&C Red No. 7 (2) and to D&C Red No. 10 (3). Since the previous report was presented at the meeting of the Association of Official Agricultural Chemists in October 1947, samples of D&C Red No. 14, D&C Red No. 34, and Ext. D&C Red No. 2 have been prepared, purified, and analyzed for one or more elements. The average purities calculated from these results were 99.9, 99.3, and 99.4 per cent, respectively. These calculated values for purity and the titanium trichloride titration figures differed by less than 1 per cent in each case.

Samples of D&C Red No. 14, Sodium Lake, D&C Red No. 34, Calcium Lake, and Ext. D&C Red No. 2, Barium Lake, were prepared and submitted to various laboratories for collaborative analysis. These lakes, prepared from the purified colors, contained about 35 per cent pure color. A portion of each of these lakes was submitted to the collaborators for analysis. The second samples submitted to the collaborators were prepared by diluting the above lakes with known amounts of talc.

Samples with directions for the analysis were sent to the following, listed alphabetically:

Ansbacher-Siegle Corporation—H. Holtzman reporting.

Calco Chemical Division American Cyanamid Company—Wm. Seaman reporting.

Harmon Color Works, Inc.—Vincent C. Vesce reporting.

Hilton-Davis Chemical Company—Anna Bartruff reporting.

H. Kohnstamm and Company, Inc.—Louis Koch reporting.

National Aniline Division, Allied Chemical and Dye Corp.—A. T. Schramm reporting.

Cosmetic Division, Food and Drug Administration—Charles Graichen and S. S. Forrest reporting.

In order to reduce the collaborators' work load to a minimum, it was requested that the results be reported in terms of ml of 0.1 *N* titanium trichloride required to titrate 0.5 gram of the lake.

The collaborative results reported last year showed considerable variation (2). It was suspected that this variation might be due to a lack of uniformity in standardization of the titanium trichloride solution. To

minimize this source of error, each collaborator was requested to standardize his titanium trichloride solution by a method supplied with the directions for the pure dye determination.¹

The results are listed in Table 1 in the order in which they were received.

Most of the collaborative results are in good agreement with one another and the calculated value. The only real difficulty was encountered with sample No. 3. Two collaborators were unable to obtain consistent values on this sample while another reported a value nearly 25 per cent below the average (collaborator No. 1). The Associate Referee encountered considerable difficulty in mixing sample No. 3. All samples were milled for several hours in a laboratory ball mill. Sample No. 3 had a marked tendency to stick to the sides of the bottle and to the balls. To insure thorough mixing, the color was scraped off frequently, but it may be that, even with this precaution, uniform mixing was not obtained. The results of six of the eight collaborators, however, are close to the calculated value.

Most collaborators reported that it was necessary to employ an indicator in the titration of samples Nos. 5 and 6. Since the use of an indicator is stated in the method as optional with the analyst, such procedure is not precluded.

Collaborative analyses have now been completed and reported on the following certifiable coal-tar color lakes:

D&C Red No. 7, Calcium Lake	D&C Red No. 31, Calcium Lake
D&C Red No. 8, Sodium Lake	D&C Red No. 34, Calcium Lake
D&C Red No. 10, Sodium Lake	Ext. D&C Red No. 2, Barium Lake
D&C Red No. 14, Sodium Lake	

The collaborative analyses show that the method is applicable to all of the colors studied. While strontium lakes have not been included in the studies thus far, it has been the experience of the color certification laboratory that they behave no differently than sodium, calcium, and barium lakes. It is, therefore, felt that they may, without collaborative study, be included in the lakes to be analyzed by the method.

The Associate Referee believes that the work done has shown the method to be reliable and convenient for the determination of pure dye in lakes of D&C Red Nos. 6-16, inclusive, 31, 34, and Ext. D&C Red No. 2.

It should be noted that not all permitted lakes of these colors have been studied collaboratively. To do so would impose an undue burden upon the collaborators. Rather, it has been the purpose of this work to study representative lakes of the more commonly certified azo colors. This phrase of the topic has now been completed.

¹ This method is described in the report of the Associate Referee on standardizations of titanium trichloride solutions (*This Journal*, p. 589) as Method II.

TABLE 1.—*Collaborative results*

COLLABORATOR	D&C RED NO. 34, CALCIUM LAKE				D&C RED NO. 14, SODIUM LAKE				EXT. D&C RED NO. 2, BARIUM LAKE			
	SAMPLE NO. 1		SAMPLE NO. 2		SAMPLE NO. 3		SAMPLE NO. 4		SAMPLE NO. 5		SAMPLE NO. 6	
	0.1 N TiCl ₄ /0.5 g	PURE DYE	0.1 N TiCl ₄ /0.5 g	PURE DYE	0.1 N TiCl ₄ /0.5 g	PURE DYE	0.1 N TiCl ₄ /0.5 g	PURE DYE	0.1 N TiCl ₄ /0.5 g	PURE DYE	0.1 N TiCl ₄ /0.5 g	PURE DYE
1	ml	per cent	ml	per cent	ml	per cent	ml	per cent	ml	per cent	ml	per cent
2	15.7		16.7		14.6*		19.5		11.5		10.7	
3	15.5		16.5		18.0		19.6		11.5		11.1	
4	15.3		16.2		—		19.1		11.1		10.7	
5	15.6		16.7		18.4		19.6		11.4		11.3	
6	15.8		16.4		18.5		19.5		12.0		11.3	
7	15.2		16.1		18.0		19.7		11.4		10.9	
8	15.4		16.4		18.3		19.4		11.3		10.8	
	15.3		16.1		18.0		20.0		11.8		10.9	
Average Value	15.5	35.7	16.4	37.7	18.2	28.6	19.6	30.8	11.5	37.6	11.0	35.9
Calculated Value	15.7	36.1	16.5	38.0	18.4	28.9	19.6	30.8	11.4	37.3	11.0	35.9
Difference	-0.2	-0.4	-0.1	-0.3	-0.2	-0.3	0	0	+0.1	+0.3	0	0

* This figure disregarded in computing the average value.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of pure dye in lakes of D&C Reds Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 31, and 34, and Ext. D&C Red No. 2 be adopted as official, first action.

(2) That the topic be continued.

REFERENCES

- (1) *Methods of Analysis*, A.O.A.C., 6th Ed. 21.41(d).
- (2) FREEMAN, KENNETH A., *This Journal*, **31**, 595 (1948).
- (3) CLARK, G. R., *Ibid.*, **28**, 761 (1945).

No report was given on buffers and solvents in titanium trichloride titrations, or on ether extract in coal-tar colors; identification of certified coal-tar colors; sulfonated amine intermediates; sulfonated phenolic intermediates; intermediates derived from phthalic acid; mixtures of coal-tar colors for drug and cosmetic use; subsidiary dyes in D&C colors; or hygroscopic properties of coal-tar colors.

The paper by K. A. Freeman and L. S. Harrow, entitled "A New Method for Determining the Boiling Range of Pseudocumidine in FD&C Red No. 1," is published in *This Journal*, **32**, 127 (1949).

The paper by M. Dolinsky, entitled "Spectrophotometric Analysis of D&C Red No. 19 (Rhodamine B)" is published in *This Journal*, **32**, 130 (1949).

* For report of Subcommittee B and action of the Association, see *This Journal*, **32**, 51 (1949).

WEDNESDAY—MORNING SESSION

REPORT ON FEEDING STUFFS

By L. S. WALKER (Vermont Agricultural Experiment Station,
Burlington, Vt.), *Referee*

RECOMMENDATIONS*

It is recommended—

(1) That further study be made on the following:

- (a) Mineral mixed feeds (calcium and iodine)
- (b) Lactose in mixed feeds
- (c) Fat in fish meal
- (d) Adulteration of condensed milk products
- (e) Crude fat of ether extract
- (f) Microscopic examinations
- (g) Fluorine
- (h) Crude fiber
- (i) Protein evaluation in fish and animal products
- (j) Hydrocyanic acid glucosides
- (k) Sampling and analysis of condensed buttermilk
- (l) Tankage (hide, hoof, horn, and hair content)

(2) It is recommended that the tentative methods for calcium and phosphorus, *This Journal*, 31, 98 (1947), and the tentative acetone method for fat in fish meal, *Ibid.*, be made official, first action.

REPORT ON MINERAL MIXED FEEDS—IODINE†

By ALFRED T. PERKINS, *Associate Referee*, and J. F. MERRILL, Kansas
Agricultural Experimental Station, Manhattan, Kans.

The Elmslie-Caldwell method as published in the 1945 A.O.A.C. *Book of Methods* is a tentative method for the determination of iodine in mineral mixed feeds. Correspondence has indicated there are questions regarding the accuracy of this method for various types of feeds, especially feeds high in content of organic matter. The Associate Referee has understood that the method is intended to be applicable for mineral mixed feeds low in organic matter. The method has been tested as a method for high mineral feeds, and no attempts have been made to adapt the method for organic feeds. A not very recent publication¹ reports extensive tests on the method, and the work of the current year has been largely devoted to re-checking the method and ascertaining the effect of variations in the published procedure.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 42 (1949).

† Contribution No. 374, Department of Chemistry, Kansas Agricultural Experiment Station.
¹ *This Journal*, 21, 597 (1938).

The ashing time and temperature has been found satisfactory for all mineral mixtures tested, but is not adequate for feeds high in content of organic matter. The recommended time and temperature is insufficient to ash organic feeds so a rapid filtration can be made.

The method has been tested to learn the effect of additions of excess bromine, and results show that such excesses do not interfere with analytical results. Sufficient bromine must be added to oxidize the iodine to iodate, and the excess bromine demands only a longer boiling time to removed this excess.

The boiling time has been tested and no loss of iodine has been found to occur with prolonged boiling.

Tests have been made on the effect of varying the amount of phosphoric acid required, and the work of 1938 has been checked. One hundred per cent of the iodine has been recovered with phosphoric acid additions of 1 ml in addition to the amount required to reach the methyl orange end-point. Larger additions of phosphoric acid do not interfere with the titration, but smaller additions will not return all of the iodine and will result in an unstable end point.

There is an indication that cooling the solution to 10° gives a minor increase in the iodine titration. However, studies of the effect of the temperature of the solution during titration fail to show a significant difference in results due to cooling.

It is recommended that new collaborative tests be made looking to the adoption of the Elmslie-Caldwell method as official.

REPORT ON THE ACTIVITY OF YEAST

By H. C. SCHAEFER (Manager, Nutrition Research Laboratories, Ralston Purina Company, St. Louis, Mo.), *Associate Referee*

Two years ago a rather detailed report of our work on the activity of yeast was made.¹ Since that time, in trying to get further information regarding current interest in yeast added to feed for fermentation, it was found that several of the large producers have discontinued production of yeast for this purpose, and that yeast of this type is being sold by a relatively small number of producers.

The addition of live, dry yeast to feeds, and allowing it to ferment, is not a practice to be encouraged or recommended. From our present knowledge of nutrition, it appears that this practice destroys carbohydrates, and apparently does not create any other nutrients to compensate for that loss; hence it does not appear to be an economical operation. In our previous work it was found that yeast, with aging, loses its viability.

¹ *This Journal*, 30, 599 (1947).

In view of the fact that there is little interest, and nothing definite to measure, it has been recommended that this work be discontinued, and that no more work be done until it is demonstrated that yeast activity creates some measurable nutrient; and that in the meantime yeast be considered in feeds merely on the basis of its value as a source of protein and water-soluble vitamins.

REPORT ON MINERAL CONSTITUENTS OF MIXED FEEDS

SAMPLE PREPARATION FOR CALCIUM AND PHOSPHORUS IN FEEDS USING NITRIC-PERCHLORIC ACID

By J. L. ST. JOHN (*Associate Referee*) and EDITH ENG HUEY (Division of Chemistry, Agricultural Experiment Stations and State Chemist's Laboratory, Pullman, Washington)

This is a continuation of the work reported in 1947 and 1948. The methods are those described in *This Journal*, 30, 606 (1947).

Two different commercial mixed feeds were secured from a commercial mill.* Sample No. 4 is a mixed ration labeled Calf Meal and sample No. 5 is a Dog Meal. One basis of selection was the fact that the Dog Meal was higher in calcium and phosphorus than the Calf Meal. The ingredients were such as are found in commercial feeds of this type, including some fortification with vitamins and minerals.

The samples were somewhat finely ground when received. They were thoroughly mixed and sampled, but purposely sent out without further

TABLE 1.—*Collaborative results*

COLLABORATORS	CALCIUM IN FEEDS				PHOSPHORUS IN FEEDS			
	SAMPLE 4		SAMPLE 5		SAMPLE 4		SAMPLE 5	
	A.	B.	A.	B.	A.	B.	A.	B.
	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	
W. R. Flach		1.18		2.36		0.75		1.49
C. Tyson Smith		1.12		2.34		0.71		1.45
W. C. Geagley	1.17	1.13	2.18	2.15	0.59	0.59	1.39	1.29
George E. Grattan		1.40		2.33		0.70		1.50
Fred E. Randall	1.12	1.15	2.27	2.28	0.81	0.81	1.57	1.58
O. R. Alexander		1.07		2.52				
P. B. Curtis		1.60		3.00		0.57		1.49
M. P. Etheredge		1.47		2.39		0.74		1.49
E. E. Huey	1.29	1.34	2.58	2.58	0.72	0.72	1.49	1.49
L. V. Burns		1.56		2.99		0.81		1.65
Averages		1.30		2.49		0.71		1.49

A.—A.O.A.C. Method.

B.—Nitric-perchloric Method.

* Centennial Flouring Mills, Spokane, Washington.

grinding. The collaborators were requested to further grind and mix before analyzing. The results are reported in Table 1. Only three collaborators determined calcium and phosphorus by the A.O.A.C. method.

The results of the different collaborators are in fairly good agreement, although it might be anticipated that the agreement would not be quite as close as would have been obtained had the samples been more finely ground by the Associate Referee. Perhaps more important are the cases where a comparison is possible. There is excellent agreement between the results obtained by the A.O.A.C. method and by the nitric-perchloric acid method of sample preparation.

Based on three years' results it is recommended† that the nitric-perchloric acid method of sample preparation be made official, first action.

REPORT ON CRUDE FIBER

By VAN P. ENTWISTLE and WM. L. HUNTER (*Associate Referee*) (Feed Control Laboratory, California Department of Agriculture, Sacramento, California)

Crude fiber is the result of an arbitrary method of treatment of a material and does not represent a definite measure of a specific substance or group of substances. Crude fiber consists largely (97%) of cellulose and lignin (1). It does not represent all of the cellulose and lignin initially present. Cellulose recovery in crude fiber is approximately 60–80%, and in lignin recovery, 4–67%. Considerable variation in the lignin content of the crude fiber fraction is found. Highly lignified materials do not necessarily yield crude fiber fractions high in lignin. Thus the crude fiber fraction obtained does not bear any definite relationship to the structural constituents of a material.

Since the crude fiber method is definitive and does not measure a fixed portion of the materials tested, it can be changed by changing the definition, with the consent of those involved. In order for a new method or modification to gain sufficient support to be adopted, it must present improvements over the present method in that it will be quicker, easier to carry out, more reproducible, or represent the structural constituents of the material more truly. It is, of course, desirable that the results obtained be comparable to those of the present official A.O.A.C. method in order to retain the value of our present data.

The present official method is based on a method developed nearly a century ago by Henneberg, Stohmann, and Rautenberg (2) in the agricultural experiment station at Weende bei Göttingen in Germany. Only slight modifications have been made in the Weende method since its development. There have been numerous attempts to develop other methods which were easier or more exact.

† For report of Subcommittee A and action of the Association, see *This Journal*, 32, 43 (1949).

A number of investigators have been of the opinion that we should attempt to approach pure cellulose and they have developed methods and evaluated them to this end.

Schulz (3) in 1856 developed a method for freeing cell membranes from encrusted lignin with nitric acid and potassium chlorate.

König (4) used glycerin and sulfuric acid to determine crude cellulose which some investigators look upon as crude fiber. This method was modified by Bellucci (5) to widen its application.

Hladík (6) used CCl_4 extracted samples and digested first with nitric and acetic acids. After pouring into cold water and washing by decantation the sample is heated with dilute sulfuric acid.

Scharrer and Kürscher (7) developed a method especially for feeding stuffs for which they claimed a higher purity for the end product (freer from lignin and pentosans). It is a single step method refluxing the sample with glacial acetic acid, nitric acid, and tri-chloroacetic acid. Since lignin is removed more extensively than in the Weende (official) method, the crude fiber values found are lower. It is claimed to be more reproducible than the Weende method due to lignin removal.

Tang, Yen, and Hsü (8) developed a method using 1.5% chlorindioxide solution, then treatment with 30% pyridine solution, and allowing the sample to stand overnight in a sodium hydroxide (7%)–sodium chloride (3%) mixture.

Other investigators have taken the view that crude fiber should be directly relative to the indigestible matter in a material. Therefore, enzymatic digestive methods were developed which purport to give a much closer correlation to the digestibility of the material under question.

Remy (9) used dilute pepsin-hydrochloric acid solution, then malt diastase solution, and finally digestion with pancreatin-sodium carbonate solution, all operations carried out at 37°C.

Horwitt, Cowgill, and Mendel (10) modified the procedure using a special takadiastase (clarase) for the malt diastase and trypsin for pancreatin. This method took 6 days to complete.

Woodson and MacKenzie (11) simplified the procedure for application to cereals. This method called for boiling the sample with water to gelatinize the starch, cooling to 38°C., digestion with pangestin for 48 hours; adjustment of the pH to 7.5–8.0, and an additional 48-hour digestion with pangestine.

All of the enzymatic methods give considerably higher results than are obtained with the Weende method. Also these methods are very lengthy and do not meet the need for a speedy analysis which most laboratories require.

The term fiber in plant materials is commonly applied to cellulose; yet it appears that crude fiber values would be more valuable as an indicator of the material's feeding value if the crude fiber contains as much

as possible of both the cellulose and the lignin. This would eliminate those methods which are attempting to approach pure cellulose. The enzymatic methods, while claiming a close correlation to the feeding value, are too slow to be of value in control work. Until a method is developed to meet the above requirements and be practical for control work, we should continue attempts to improve our present method.

The A.O.A.C. method has been reexamined at intervals with numerous helpful criticisms, but with no basic changes. Bidwell and Walton (12), Bidwell and Bopst (13), Francis (14), Hanson (15), and others have studied thoroughly the various steps in our present official method. The results of their findings are:

- (1) Size and shape of flask are not critical so long as the material does not adhere to sides of flask out of digestion solution.
- (2) Any efficient condenser is satisfactory.
- (3) 350-mesh wire filters gave results comparable to cloth filters.
- (4) Non-fat extracted samples gave slightly higher results.
- (5) Volume of digestion solution not critical. 400 ml of solution did not lower results appreciably.
- (6) The fineness of grind affects results. The finer the sample is ground the lower the crude fiber result.
- (7) Neutralization of the acid, rather than filtering off, may give high results because of precipitation of substances previously made soluble in the acid digestion.
- (8) Intensity of heat applied affects results. Vigorous boiling gives lower results than a less violent boiling.
- (9) Filtering aids showed little effect. Asbestos caused slightly lower results on some materials, slightly higher on others.
- (10) Delay in filtering. A delay of 7 minutes in the acid filtration will cause a lower result due to the continued action of the acid. A similar delay in the alkali filtration has the same effect for the same reason at first, but if the delay continues, substances made soluble in the hot alkali may precipitate as the solution cools. This is especially true of samples high in protein. Neubert, Van Amburgh, and St. John (16) found that samples which filtered difficultly in the final filtration could be made to filter rapidly by the addition of potassium sulfate. Results of this method agree very closely to the official method on easily filtered samples, but give somewhat lower values on those which filter slowly. However, it is felt that this modification yields results more valid than the official method, because the long time involved for the final filtration introduces errors in the official method.

The official method (17) allows latitude in the size and type of digestion flask, type of condenser, filtering cloth and in the choice of Gooch or alundum crucibles for the final filtration. It specifies the use of asbestos as a filter aid. A survey conducted by Hunter (18), of 69 participants in the American Association of Feed Control Officials collaborative work, shows that the various laboratories vary considerably on these optional points and also on others where no option is allowed. This is done in spite of the necessity of close adherence to an empirical method, as stated by Bidwell and Walton (12), Hunter (18), and others. A study of the results obtained in this collaborative series does not show however, that there is any

correlation between results obtained and equipment and technique used. Of the 69 participants surveyed, only seven were found who were using the same method throughout the determination. The survey showed the following types of equipment and technique used:

TABLE 1.—*Equipment and technique of 69 collaborators*

	Digestion Vessel Size (ml)						
	300	400	500	600	750	800	1000
Number using	1	1	23	20	10	1	13
	Beaker, Tall form			Vessel Shape Beaker, Regular		Flask, Erlenmeyer	
	37		9		23		
	Cloth Filter		First Filtration Wire Filter		Neutralization Other Types Filter		
	55		6		3		4
	Gas			Source of Heat			
	27			Electric			
	Air		Type of Condenser Running Water			Flasks Filled with Water	
	9		58			2	
	Asbestos		Filter Aid Glass Wool			None	
	34		1			33	
	Gooch		Final Filtration Alundum			Other	
	38		27			3	

This shows clearly that 500 or 600 ml tall form beakers are the choice of the majority. Cloth filters for the acid filtration, electric heat, and running water condensers are wide favorites. Asbestos, while specified in the official method, is used by approximately only half of those surveyed. Gooch crucibles are a slight favorite for the final filtration. It is interesting to note that of the 38 using Gooch crucibles, 22 (58%) used asbestos,

whereas of the 27 using alundum crucibles, only 6 (22%) used asbestos. We have found that asbestos speeds the final filtration if the proper type of asbestos is used. All asbestos is not satisfactory, even so-called Gooch grades. Bakers "Powminco" grade acid washed and fired is the only type which we have found that is satisfactory. Unreported variations are doubtless being made which also have their effect on the crude fiber value found.

One point that the A.A.F.C.O. collaborative work demonstrates is the fallacy of reporting crude fiber results to the second decimal place. A study of the crude fiber results of this collaborative work indicates that for the present, crude fiber results should not be reported closer than to the nearest quarter per cent. Using the crude fiber average and allowing a tolerance of one quarter per cent, about one third, at best, of the collaborators will still be outside of the range. To continue to report to the second decimal place is to continue to place an absolutely false value on the reliability of the result found. Reporting to the closest quarter per cent will still leave many laboratories who will need to improve their technique and follow the official method more closely in order to fall within the suggested tolerance.

Popov (19) and Lepper (20) have suggested radical changes in the Weende (A.O.A.C.) method. Popov reports that by increasing the concentration of the acid and alkali digestion solutions to 2.1% the digestion

TABLE 2.—*Comparative data on time of digestion*

TYPE OF MATERIAL	LEPPER METHOD	A.O.A.C. METHOD
	10 MIN.	30 MIN.
	<i>per cent</i>	<i>per cent</i>
Broiler feed	5.9	5.7
Rabbit pellets	6.9	6.3
Meat and bone scraps	2.9	2.5
Dairy feed	8.1	8.2
Turkey starter mash	6.0	5.8
Turkey feed	6.6	6.3
Turkey feed	7.0	6.7
Turkey finish mash	6.0	5.8
Dairy feed	8.0	7.0
Ground barley	6.9	6.7
All purpose mash	5.8	5.6
Calf meal	7.7	7.8
Meat and bone scraps	1.3	1.6
Cottonseed meal	9.7	10.0
Ground barley	7.6	7.7
Corn gluten meal	4.2	4.1
Wheat bran	9.6	10.0
Copra	9.5	10.3
Alfalfa meal	26.8	27.8

time can be shortened to 15 minutes, and still yield results comparable with the usual 30-minute digestion with 1.25% solutions. Lepper advocates using 3.125% acid and alkali solutions and shortening digestion time further to 10 minutes. He also claims results comparable to the Weende (A.O.A.C.) method. Lepper tried to shorten the digestion time to 5 minutes by further increasing the concentration of the digestion solutions, but it was not satisfactory. We have made a short investigation of Lepper's method. The results obtained are shown in Table 2.

These data definitely indicate a possibility of shortening the time required for the determination without disturbing present concepts of crude fiber.

It appears that Lepper's method or that of Popov may at least have a place in industrial control work where results are needed in a hurry. The data also suggests that laboratories located at high altitudes might vary the digestion solution concentration or digestion time to compensate for reduced boiling temperatures.

It is strongly urged that one or both of these shorter methods be given collaborative study to determine their merit as a possible replacement for the present official method.

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No reports were given on lactose in mixed feeds, fat in fish meal, adulteration of condensed milk products, crude fat or ether extract, microscopic examination, fluorine, protein evaluation in fish and animal products, hydrocyanic acid glucosides, sampling and analysis of condensed buttermilk, and tankage (hide, hoof, horn, and hair content).

No report was given on waters, brine, and salt, including boron in water and fluorine in salt.

CORRECTIONS IN FEBRUARY JOURNAL

Report on Changes in Methods, Vol. 32, No. 1

Page 74. Pyrogallol in Hairdyes, Quantitative determination: Under "reagents," line 2, change " FeDo_4 " to read " FeSO_4 ."

Page 75. Line 18. Delete "s" on "*Solutions*." Line 3. Change "*Aluminum*" to "*Alumina*." Under "Standardization," change formula to read " $k = \frac{D}{c}$ " (Insert = sign). Under Liquid Dyes "Continuous Extraction," line 11, change "in" to "into."

Page 76. Henna Powder Mixture, line 1, after "Weigh 0.9 to 1.1" insert "g."

ANNOUNCEMENT

L. M. Beacham, Food and Drug Administration, Washington, D. C., has been appointed Referee on Processed Vegetable Products, in place of V. B. Bonney.

CONTRIBUTED PAPERS

THE ANALYSIS OF CASTOR OIL IN LIPSTICK*

By S. H. NEWBURGER (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

A principal ingredient of nearly all lipsticks, castor oil consists essentially of the glyceryl ester of ricinoleic acid, an unsaturated hydroxy fatty acid. The most widely used analytical methods for the determination of castor oil employ the acetylation of the free hydroxyl group as the basis of the analysis. Modern lipsticks, however, contain many substances in addition to castor oil which can be acetylated. Beeswax has an acetyl value of 15.2, carnauba wax 55.2, and lanolin 23. Most of the organic lakes, fluorescein dyes, and oil soluble colors have active hydroxyl or amino groups. Still other easily acetylated materials which may be encountered are glyceryl monostearate and cetyl alcohol. It is obvious, therefore, that the application of the acetylation technique to the determination of castor oil in lipstick requires the preliminary removal of interfering substances.

The following is a brief summary of the method developed: The lipstick was boiled with benzene, centrifuged, the liquid decanted, and the residue of insoluble lakes discarded.¹ The benzene was evaporated, the residue treated with hot glacial acetic acid and with hot light mineral oil. Most of the organic material was extracted into the mineral oil layer; while the castor oil, together with some of the dyes and other materials, was drawn off in the glacial acetic acid. The glacial acetic acid was diluted with water and extracted with ether. The ether solution in turn was extracted with dilute aqueous alkaline alcohol to remove fluorescein dyes. Evaporation of the ether left a residue which consisted of the castor oil, oil soluble color, and small amounts of other substances. The residue was saponified and the unsaponifiable matter discarded. This eliminated the oil soluble dye, any higher alcohols, and entrained mineral oil. Finally, the fatty acids including those of the castor oil were extracted. By acetylation of these acids it was possible to determine the castor oil content of the lipstick. A more detailed account of the procedure is as follows:

MATERIALS USED IN THE PREPARATION OF THE EXPERIMENTAL LIPSTICKS

- (1) *Lipstick base*.—A castor oil-free lipstick base was prepared. It is doubtful whether such a base is of practical value; however, it was desired to incorporate a number of ingredients which would interfere with the acetylation procedure. The following ingredients were melted together at 100°C and then cooled to room temperature to form the experimental lipstick base:

* Presented at the Annual Meeting of The Association of Official Agricultural Chemists, held at Washington, D. C., October 11, 12, and 13, 1948.

¹ The method used for the elimination of the lakes was suggested in a private communication from K. A. Freeman of this Division.

	Gms.
U.S.P. White Beeswax.....	30
U.S.P. Anhydrous Lanolin.....	10
U.S.P. Heavy Mineral Oil.....	5
U.S.P. Petrolatum.....	5
Ceresin.....	5
Glyceryl Monostearate.....	5
Carnauba Wax.....	5
Cetyl Alcohol.....	3

(2) *Lake mixture*.—A combination of certified lakes was prepared:

Lake	% Pure Dye	Substratum	Gms.
D&C Red No. 9 Ba Lake.....	36	Gloss White	2
D&C Red No. 10 Na Lake.....	83	Gloss White	2
D&C Red No. 31 Ca Lake.....	44	Gloss White	2
D&C Orange No. 15 Ca Lake.....	35	Gloss White	2

(3) *D&C Red No. 21*.—A certified straight color containing 98% dye.

(4) *D&C Red No. 17*.—A certified straight color containing 94% dye.

(5) *Castor Oil*.—A U.S.P. product.

The various experimental lipsticks were prepared by heating together at 100°C., lipstick base, lake mixture, D&C Red No. 21, D&C Red No. 17, and castor oil.

METHODS OF ANALYSIS

PROCEDURE

Isolation of castor oil fatty acids:

Weigh ca 4 gm of lipstick into a 250 ml beaker, add 50 ml of benzene, cover beaker with watch-glass, and heat to boiling for several minutes. Cool the mixture to room temperature and transfer to centrifuge tube. Rinse the original beaker with 10 ml of hot benzene and add to the contents of the centrifuge tube. Centrifuge mixture and decant liquid thru cotton plug into a 150 ml beaker. Add 25 ml of hot benzene to residue in centrifuge tube, mix thoroly, again centrifuge and also decant into the 150 ml beaker. Evaporate the benzene on the steam bath, add 10 ml of glacial acetic acid to the residue, cover beaker with watch-glass, boil for several minutes, and transfer the hot mixture to a 250 ml separatory funnel. Rinse beaker with 10 ml of hot glacial acetic acid and 30 ml of hot (100°C.) U.S.P. light mineral oil and add rinsings to the contents of the separatory funnel. Shake mixture well.* Fasten stopcock of separatory funnel with a rubber band, partially immerse the separatory funnel in a steam bath for several minutes and then draw off the acetic acid layer into a 100 ml beaker. Continue the extraction with 2 additional 20 ml portions of hot glacial acetic acid taking care to keep the mixture hot by immersing the separatory funnel in the steam bath for a few minutes each time. Combine the acetic acid extracts, cool to room temperature with stirring, and allow to stand for $\frac{1}{2}$ hour. Slowly add 3 ml of water with stirring and allow to stand for another five minutes. Filter the mixture slowly by gravity or gentle suction thru a Gooch crucible containing a $\frac{1}{4}$ " to $\frac{1}{2}$ " layer of sand over an asbestos mat. Wash the filter with a solution of 15 ml of acetic acid and 1 ml of water. Transfer the filtrate and washings to a 500 ml separatory funnel with the aid of 100 ml of ether, add 250 ml of water and shake well. Draw off the aqueous layer and re-extract with 2 additional 50 ml aliquots of ether. Wash the combined ether extracts with two 50 ml portions of water. Discard washings and extract the ether with two 100 ml portions of a 20% alcohol solution containing 3% KOH. Continue the extraction with 50 ml portions

* In extracting hot mixtures a cotton glove was worn to protect the hand.

of the alkaline alcohol solution until any extracted color shows little or no fluorescence. Discard the alkaline extracts, acidify the ether solution with HCl, wash with two 50 ml portions of water, and evaporate the ether on the steam bath. Saponify the residue by refluxing for 2 hours with a mixture of 50 ml of benzene, 25 ml absolute alcohol, and 1 gm KOH. Transfer the hot saponified mixture to a separatory funnel, add 50 ml of hot water and shake well.³ Draw off the aqueous layer and extract with 2 additional 20 ml portions of hot benzene. Wash the combined benzene extracts with two 25 ml portions of 30% alcohol containing 1% KOH. Discard the benzene solution. Combine the two alkaline alcohol wash solutions, extract with 20 ml of ether to remove dye, discard the ether, and add the alkaline wash solution to the original extracted alkaline aqueous solution. Acidify the alkaline solution and extract with 30, 20, and 20 ml portions of benzene. Combine the benzene extracts, wash with water, and filter thru a cotton plug into a tared beaker. Evaporate the benzene on the steam bath, dry residue at 100°C. for 10 minutes in oven, cool, and weigh as fatty acids.

Acetylation of the hydroxy fatty acids:

Dissolve the isolated fatty acids in benzene, transfer to a 50 ml volumetric flask, dilute to mark with benzene, and mix. Pipet a 25 ml aliquot into a 125 ml acetylation flask and reserve the remaining solution for a blank. Evaporate the benzene on the steam bath with the aid of a glass tube, connected to the vacuum, and projecting into the acetylation flask. Add 5 ml of acetic anhydride and reflux for 2 hours. Add 25 ml of hot water, allow to stand on steam bath for 30 minutes with occasional swirling, add 15 ml of toluene, and transfer the hot mixture to a 250 ml separatory funnel. Rinse the acetylation flask with 25 ml of toluene and add rinsings to the contents of the separatory funnel. Fasten stopcock of separatory funnel with a rubber band, partially immerse the separatory funnel in the steam bath for a few minutes, and then shake well for about five minutes. Again partially immerse the separatory funnel in steam bath for a few minutes and then draw off the aqueous layer. Continue the extraction with 15 ml portions of hot water, heating each time in the steam bath, until the aqueous layer requires less than 0.1 ml of 0.1 N NaOH to turn phenolphthalein pink. Filter the toluene solution thru a cotton plug into a tared 150 ml beaker, and wash the separatory funnel and cotton plug with benzene. Evaporate the toluene-benzene solution on the steam bath, dry the residue at 100°C. for 15 minutes in an oven, cool, and weigh the acetylated material.

Transesterification of the acetylated material and subsequent saponification of the ethyl acetate:

The procedure is based on the Sclar and Clark⁴ modification of Freudenberg's method. This is an indirect method as the acetyl group of the acetylated hydroxy acids is converted into ethyl acetate which is in turn saponified.

The apparatus is described in *This Journal*, 27, 473 (1944).

Surround the receiver with an ice bath.

Dissolve and transfer the acetylated material to the distilling flask with 20 ml of hot absolute alcohol. Dissolve and transfer 2 gm. of *p*-toluenesulfonic acid to the same flask with another 10 ml of absolute alcohol.

Distill from a vigorously boiling water bath; 20 minutes after the first distillate drops into the receiver, add 25 ml of absolute alcohol and distill for 20 minutes; then add another 25 ml of absolute alcohol and distill for another 20 minutes.

Remove the boiling water bath, disconnect the receiver, stopper and label as

³ Troublesome emulsions can be broken by fastening the stopcock of the separatory funnel with a rubber band and partially immersing the separatory funnel in a steam bath until the contents begin to boil.

R. N. Sclar and G. R. Clark, *This Journal*, 27, 472 (1944).

distillate No. 1. Connect another receiver, replace boiling water bath, add 25 ml absolute alcohol to the distilling flask and distill for 20 minutes. Disconnect the receiver and label the distillate No. 2.

Add 25 ml of 0.1 *N* NaOH to distillate No. 1 and 10 ml of 0.1 *N* NaOH to distillate No. 2, connect the receivers to reflux condensers, and immerse the flasks in a boiling water bath for 15 minutes. Wash down the condenser of receiver No. 1 with 50 ml of water and that of No. 2 with 15 ml of water. Cool the two solutions to room temperature, add 5 drops phenol red indicator (0.1% alcohol solution) to distillate No. 1 and 3 drops to distillate No. 2. Titrate with 0.1 *N* HCl to a yellow end point.

Standardize the 0.1 *N* HCl against 0.1 *N* NaOH by pipeting 25 ml of the standard alkali into a solution of 75 ml of absolute alcohol and 50 ml of water. Add 5 drops of phenol red indicator solution and titrate with the HCl to a yellow end point. Calculate the normality of the HCl.

Run a blank on the reserved unacetylated fatty acids as follows: Transfer the approximately 25 ml of reserved benzene solution to the distillation flask, evaporate the benzene, and add 2 gm *p*-toluenesulfonic acid and 30 ml of absolute alcohol. Proceed as described for the acetylated material beginning with: "Distill from a vigorously boiling water bath . . ."

Calculation of per cent castor oil.—Subtract the HCl titers of the acetylated material from the corresponding titers of the blank and add the two differences. Use this value in the following formula to calculate the percentage of castor oil.

$$\frac{\text{Ml of 0.1 N HCl} \times 0.004001 \times 2 \times 100}{0.1177 \times 0.951 \times \text{w't of sample (gm)}} = \% \text{ Castor Oil.}$$

Ml of 0.1 *N* HCl \times 0.004001 = gm of NaOH required to saponify the acetylated material.

0.1177 = gm of NaOH required to saponify compounds obtained from the acetylation of 1 gm of castor oil fatty acids; see Table 2.

0.951 = fatty acid fraction of castor oil; see Table 1.

The factor of 2 is used because only one-half of the sample is acetylated.

EXPERIMENTAL RESULTS

Castor oil was saponified and the unsaponifiable matter and fatty acids determined. The procedure was similar to that already described with the exception that chloroform instead of benzene was used to extract the fatty acids. The results are given in Table 1.

TABLE 1.—*Saponification of castor oil*

SAMPLE U.S.P. CASTOR OIL	UNBAPONIFIABLE MATTER	FATTY ACIDS
gm	gm	gm
3.462	0.019 (0.5%)	3.291 (95.1%)

The castor oil fatty acids were acetylated, transesterified, and the ethyl acetate saponified according to the described procedure. The data are given in Table 2.

It should be noted that the saponification values in Table 2 are based on

TABLE 2.—*NaOH required to saponify acetylated castor oil fatty acids*

SAMPLE CASTOR OIL FATTY ACIDS	WEIGHT OF ACETYLATED MATERIAL	NaOH REQUIRED TO SAPONIFY ACETYLATED ACIDS
gm	gm	gm NaOH/gm fatty acids
0.414	0.453	0.1167
0.211	0.232	0.1187
		Av. 0.1177

TABLE 3.—*Analysis of castor oil in lipstick*

SAMPLE		WEIGHT OF FATTY ACIDS	WEIGHT OF ACETYLATED MATERIAL	CASTOR OIL	
				ADDED	FOUND
	gm	gm	gm	per cent	per cent
Lipstick base	3.500	0.138	0.069	None	0.8
Lake mixture	0.400				
D&C Red No. 21	0.080				
D&C Red No. 17	0.020				
U.S.P. castor oil	0.000				
Total	4.000				
Lipstick base	3.300	0.350	0.183	5.5	6.2
Lake mixture	0.400				
D&C Red No. 21	0.080				
D&C Red No. 17	0.020				
U.S.P. castor oil	0.221				
Total	4.021				
Lipstick base	3.100	0.611	0.329	12.5	13.3
Lake mixture	0.400				
D&C Red No. 21	0.080				
D&C Red No. 17	0.020				
U.S.P. castor oil	0.515				
Total	4.115				
Lipstick base	2.700	0.871	0.472	20.2	20.1
Lake mixture	0.400				
D&C Red No. 21	0.080				
D&C Red No. 17	0.020				
U.S.P. castor oil	0.811				
Total	4.011				

the original weight of the fatty acids rather than the weight of the acetylated product.

Lipstick samples varying in castor oil content were prepared and analyzed by the outlined method of analysis. The results are given in Table 3.

DISCUSSION

The data shown in Table 3 indicate that the castor oil content of a lipstick can be determined within 1%. As there is a blank of 0.8% on the castor oil free ingredients, the values should be high. This was observed with two of the experimental lipstick preparations. With the third preparation, highest in castor oil content (20.2%), approximately the theoretical result was obtained. A loss of some castor oil was probably counterbalanced by the blank.

If one assumes that all the castor oil fatty acids have been recovered, then, from the data in Table 3, the weight of non-castor oil fatty acids varies from 0.100 gm to 0.140 gm. It is conjectured that most of this material is contributed by the glyceryl monostearate.

It is unfortunate that the final titers are rather small. The compounds obtained from the acetylation of one gram of castor oil fatty acids require only 29.4 ml of 0.1 *N* NaOH for saponification. The blank on the unacetylated material never exceeded 0.3 ml of 0.1 *N* NaOH. This blank is, therefore, of doubtful necessity when only a rough estimate is required of the castor oil present.

As it is not possible to easily obtain two 25 ml aliquots from 50 ml of solution, the blank contains a little less material than the aliquot taken for acetylation. However, this difference is not significant since the blank is small.

CONCLUSION

An analytical method has been developed for the determination of castor oil in lipstick. Typical results indicate that the castor oil can be determined to within 1%.

THE ASSAY OF PROCAINE HYDROCHLORIDE SOLUTION, N.F.

By SAM D. FINE, Food and Drug Administration, Federal
Security Agency, Cincinnati, Ohio)

The September, 1948, issue of the *Journal of the American Pharmaceutical Association* contains a paper (1) on the spectrophotometric determination of procaine in procaine penicillin G. The method is quite simple, involving merely the weighing of a sample, making two dilutions, and measuring optical density at 290 $m\mu$ against water as a blank. The

concentration of the solution is obtained by calculation from an extinction coefficient determined on a sample of pure procaine hydrochloride.

If there is no interference from the other ingredients, it should be possible to assay procaine hydrochloride solution, N.F., by diluting to a suitable concentration with water, measuring the optical density against a water blank and calculating the concentration from an extinction coefficient as above. The National Formulary product contains 20 mg of procaine hydrochloride per milliliter in isotonic salt solution; various preservatives may be used, including chlorbutanol, cresol, and sodium bisulfite.

EXPERIMENTAL

Apparatus.—Transmittancy measurements were made with a Beckman quartz spectrophotometer equipped with one centimeter silica cells and ultraviolet radiation source operating with a spectral band width of approximately 1–5 $m\mu$.

Effect of Interferences.—The effect of the interference by sodium chloride, chlorbutanol, cresol, and sodium bisulfite was investigated. A solution of each was prepared of the same concentration that is present upon dilution of the N.F. product as directed by the recommended spectrophotometric procedure. Transmittancy measurements were made on these solutions from 220 to 320 $m\mu$. The results are shown in Table 1. Transmission of all is greater than 95% at 290 $m\mu$.

TABLE 1.—Transmission (per cent) of sodium chloride, sodium bisulfite, chlorbutanol, and cresol

WAVE LENGTH	SODIUM CHLORIDE	SODIUM BISULFITE	CHLORBUTANOL	CRESOL
$m\mu$	0.8 mg/1000 ml	0.8 mg/1000 ml	1.0 mg/1000 ml	0.8 mg/1000 ml
220	91.5	94.7	89.2	87.0
230	93.6	96.5	92.5	93.5
240	94.9	97.7	94.3	96.9
250	95.5	98.5	95.0	97.5
260	96.0	98.5	95.2	97.2
270	96.2	98.5	95.5	97.3
280	96.4	98.8	95.8	97.3
290	96.8	98.8	96.1	97.4
300	97.0	98.4	96.2	97.7
310	97.2	98.3	96.5	98.2
320	97.3	98.6	96.7	98.4

Comparison of Spectrophotometric and National Formulary (2) Methods.

—A solution was prepared containing 20 mg/ml of procaine hydrochloride and 5 mg/ml of chlorbutanol in isotonic salt solution. Transmittancy measurements from 220 to 320 $m\mu$ made on a dilution of this solution disclosed that maximum absorption was at 290 $m\mu$. Assay by the National Formulary method gave 19.8 mg/ml; assay by the spectrophotometric

method gave 20.2 mg/ml. A series of samples from different manufacturers were assayed by the spectrophotometric procedure and the National Formulary method. The results are shown in Table 2.

TABLE 2.—*Comparison of spectrophotometric method and National Formulary method*

SAMPLE	PRESERVATIVE	PROCAINE HYDROCHLORIDE, MG/ML	
		SPECTROPHOTOMETRIC METHOD	N.F. METHOD
1	Chlorbutanol	21.4	21.3
	Sodium bisulfite		
2	Sodium bisulfite	19.8	20.0
	Chlorbutanol		
3	Chlorbutanol	19.5	19.8
	Sodium bisulfite		
4	Sodium bisulfite	19.9	19.7
	Chlorbutanol		
5	Chlorbutanol	20.7	20.4
	Sodium bisulfite		
6	Sodium bisulfite	20.2	19.9
	Chlorbutanol		
7	None	20.8	20.4
	Chlorbutanol		
8	Chlorbutanol	20.3	20.4
	Cresol		
9	Cresol	20.1	19.8
	Chlorbutanol		
10	Chlorbutanol	20.0	19.9
	Chlorbutanol		

Effect of Decomposition of Procaine Hydrochloride: Spectrophotometric Assay.—One sample assayed by the recommended spectrophotometric procedure was found to contain less than the declared amount of procaine hydrochloride. Assay by the National Formulary method gave erratic results. Assay was made by A.O.A.C. Methods, 39.102 (3) (bromination) and 39.104 (3) (distillation). Both methods determine total procaine originally present, and the results were 19.3 and 19.7 mg/ml, or approximately the declared amount. Procaine hydrochloride decomposes by hydrolysis into *p*-amino benzoic acid and β -diethylamino ethyl alcohol. A study of the ultra-violet absorption of procaine hydrochloride and of its decomposition products was made. Transmittancy measurements were made for each from 220 to 320 $m\mu$; the results are shown in Figure 1. The maximum absorption for procaine hydrochloride is at 290 $m\mu$; the maximum for *p*-amino benzoic acid is at 268 $m\mu$; β -diethylamino ethyl alcohol does not absorb ultraviolet light, the transmission being greater than 95% in the region where the other two compounds exhibit maxima.

Transmittancy measurements were made on the partially decomposed sample from 220 to 320 $m\mu$; the results are also shown on Figure 1. The maximum is at 286 $m\mu$, in contrast to the maximum of 290 $m\mu$ found for the prepared sample. These results indicated that both procaine hydrochloride and *p*-amino benzoic acid were present.

After extraction from ammoniacal solution with chloroform as in the National Formulary method, the solution of this sample was acidified and

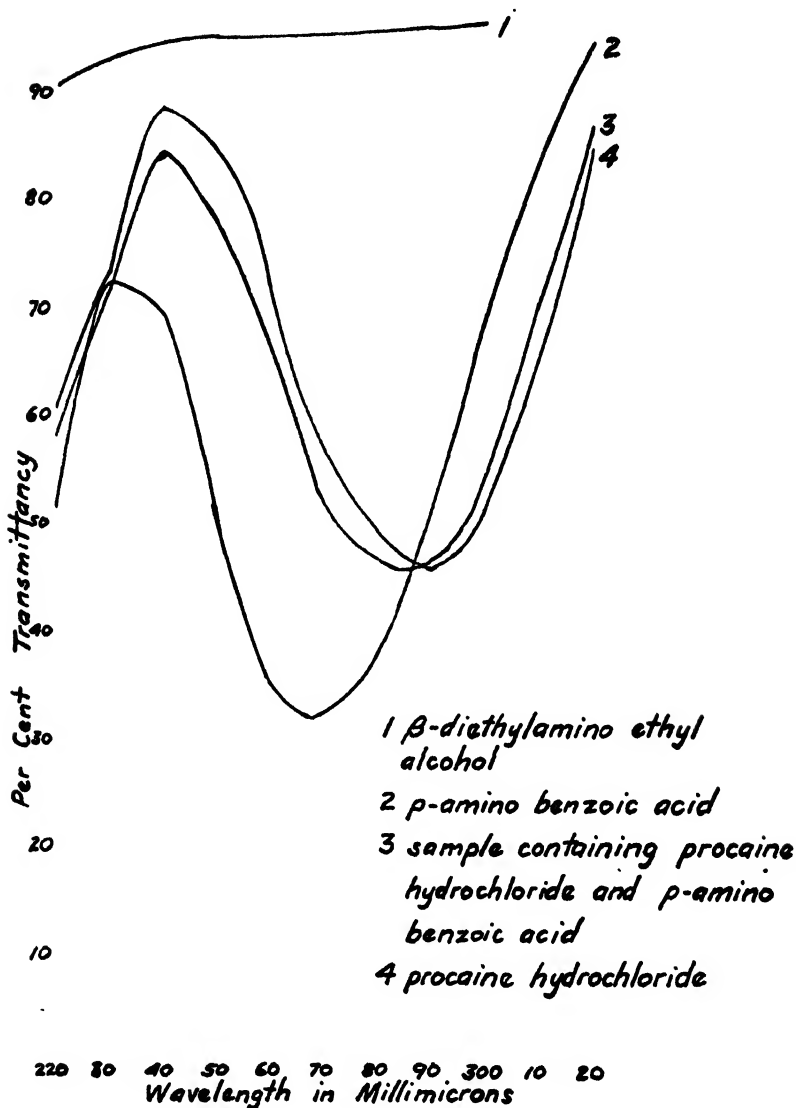


FIG. 1

extracted with ether. The extract, after evaporation of the ether, was dried and the melting point was found to correspond with that of *p*-amino benzoic acid. The *p*-amino benzoic acid was determined quantitatively by bromination after extraction of the procaine. Results were 5.3, 5.5 mg/ml of *p*-amino benzoic acid.

A spectrophotometric method of assay for both the procaine hydrochloride and the *p*-amino benzoic acid was devised. The optical densities

of standards and of the sample were measured at 268 and 290 $m\mu$, and a set of two equations in two unknowns established. Let

E_1 = extinction coefficient for procaine hydrochloride at 290 $m\mu$.

E_2 = extinction coefficient for *p*-amino benzoic acid at 290 $m\mu$.

E_3 = extinction coefficient for procaine hydrochloride at 268 $m\mu$.

E_4 = extinction coefficient for *p*-amino benzoic acid at 268 $m\mu$.

C_p = concentration of procaine hydrochloride as mg./100 ml.

C_b = concentration of *p*-amino benzoic acid as mg./100 ml.

D_{290} = optical density of the unknown at 290 $m\mu$.

D_{268} = optical density of the unknown at 268 $m\mu$.

Then

$$D_{290} \times 1000 = E_1 C_p + E_2 C_b$$

$$D_{268} \times 1000 = E_3 C_p + E_4 C_b$$

Solving for C_p and C_b

$$C_p = \frac{1000 (E_4 D_{290} - E_2 D_{268})}{E_1 E_4 - E_2 E_3}$$

$$C_b = \frac{1000 (E_1 D_{268} - E_3 D_{290})}{E_1 E_4 - E_2 E_3}$$

Using this procedure, 11.0 mg/ml of procaine hydrochloride and 4.5 mg/ml of *p*-amino benzoic acid were found. The 4.5 mg of *p*-amino benzoic acid is equivalent to 9.0 mg of procaine hydrochloride. This corresponds to a total of 20.0 mg/ml of procaine hydrochloride originally present. (The figures found for total procaine hydrochloride by the two A.O.A.C. (3) methods were 19.3 and 19.7 mg/ml.)

In order to check this method, a solution containing procaine hydrochloride and *p*-amino benzoic acid was prepared and assayed. Recovery of the added procaine hydrochloride was 97.1%, and of the added *p*-amino benzoic acid 102.0%.

Effect of Decomposition of Procaine Hydrochloride: The National Formulary Assay.—The effect of the second decomposition product, β -diethylamino ethyl alcohol was considered. It is soluble in both water and organic solvents. It is the product that is distilled over in A.O.A.C. (3) method 39.104, and if present in the residue after evaporation of the chloroform, would consume standard acid. The results obtained on the partially decomposed sample by the National Formulary method were 16.8, 13.9, 15.9, 14.1, 15.6, and 12.6 mg/ml procaine hydrochloride. Further determinations by the National Formulary method were made, with the chloroform being evaporated under varying conditions. Dependent upon these conditions, more or less standard acid was consumed. A laboratory sample of 2% procaine hydrochloride solution was assayed by the National Formulary method, and the residue after evaporation of the chloroform was allowed to remain on the steam bath for over a minute. There was no appreciable loss of procaine base, as determined by consumption of standard acid.

When applied to a partially decomposed solution of procaine hydrochloride, the procedure specified in the National Formulary monograph results in β -diethylamino ethyl alcohol being present along with procaine in the chloroform extract. The variation in the results is due to the variation in the evaporation of this volatile constituent. The National Formulary method is the same as A.O.A.C. (3) method 39.103. This latter method bears the parenthetical statement that it determines only undecomposed procaine. However, a review of the collaborative work leading to its adoption as official, failed to disclose that it was ever tried on a partially decomposed procaine hydrochloride solution.

Recommended Spectrophotometric Procedure—Pipet 10 ml of the sample into a 1000 ml volumetric flask and make to volume with distilled water. Transfer 20 ml of this dilution to a 1000 ml volumetric flask, dilute to volume with distilled water, and measure the optical density of the resulting soln at 290 $m\mu$ against water in the reference cell. Obtain the concentration of the soln by calculation from an extinction coefficient determined on a sample of pure procaine hydrochloride. Determine if any decomposition of the procaine has occurred by checking the wave length of maximum absorption. Maxima at less than 290 $m\mu$ indicate decomposition. By use of "two color" analysis, proportion of procaine hydrochloride and *p*-amino benzoic acid can be determined. (Clean absorption cells thoroly before use. Take the average of the readings for several adjustments of the instrument.)

SUMMARY

A spectrophotometric method for the assay of procaine hydrochloride solution, N.F., has been described. Accuracy, compared with the National Formulary method, is good where no decomposition of the procaine hydrochloride has occurred. The National Formulary method and the A.O.A.C. (3) method 39.103 are not applicable to decomposed procaine hydrochloride solutions. Decomposition of such solutions can be detected by determination of the wave length of maximum absorption. By the use of "two color" analysis, the extent of decomposition can be determined.

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THE ANALYSIS OF MIXTURES OF FATTY OILS WITH HYDROCARBONS

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London, England)

S. H. Newburger (1) has worked out a method, based on a statement of the author (2), for the separation of hydrocarbons from their mixtures with wool grease by chromatographing a solution of the mixture in

petroleum benzin (B.P. 30–75°C.) through an activated aluminum oxide column. Under the conditions he describes, both hydrous and anhydrous lanolin are adsorbed to an extent greater than 99 per cent; hydrocarbons are not adsorbed at all, but may be recovered quantitatively from the percolate from the column.

For more general application, especially for the determination of small percentages of mineral oil in admixture with fatty oils, it is not possible to apply the chromatographic technique directly to the oil-hydrocarbon mixture. In such cases, the unsaponifiable matter should first be prepared and, after solution in light petroleum or petroleum benzin, should be passed through the column of aluminium oxide. If suitable conditions are employed for the chromatographing, hydrocarbons percolate through the column and may be recovered from the percolate quantitatively, while the rest of the unsaponifiable matter remains adsorbed. There is abundant evidence (1, 2, 3, 4) that the percolate consists of pure hydrocarbon. The technique of S. H. Newburger (3) may be employed for the separation. The following alternative technique has been in use in the author's laboratory for a number of years.

METHOD

PREPARATION OF Al_2O_3 COLUMN

Apparatus.—Constrict a glass tube, 30 cm. to 40 cm. long, and 1.5 cm. in internal diameter, at one end and join the constricted end to a short piece of glass tubing of 0.5 cm. internal diameter. Fix the tube vertically by passing the narrower end thru a cork into a filter flask. Tamp a 1.5 cm. cotton plug into the bottom of the wide part of the tube.

Column.—Mix aluminium oxide of suitable grade (Aloreo grade F-20 mesh 80–200 or B.D.H. aluminium oxide for chromatographic analysis) with sufficient light petroleum (B.P. 40–60°C.), or petroleum benzin (B.P. 30–75°C.) to form a thin slurry from which air trapped in the Al_2O_3 separates easily. Pour the slurry carefully into the chromatograph tube thru a funnel so that it distributes itself evenly. Apply very light suction to the filter flask and, while maintaining constantly a head of petroleum over the Al_2O_3 , build up the depth of this in the tube to a total depth of approximately 15 cm. by further additions of the slurry. Maintain a layer of at least 2 cm. of petroleum over the top surface of the column from this point until the chromatography is finished.

DETERMINATION

Prepare the unsaponifiable matter from a suitable weight of the oil, quantitatively by a recognised standard method. In the case of most fatty oils, the weight of oil taken should be from 2.0 to 2.2 grams; if the percentage of unsaponifiable matter exceeds 5 per cent, the weight taken should be reduced to approximately 1 gram. The method of most general application for the quantitative separation of the unsaponifiable matter is the S.P.A. method (5); if the oil concerned in the test is not of marine origin, use either the method of the A.O.C.S. (6) or that of Bolton and Williams (7).

Dissolve the unsaponifiable matter so prepared in from 50 to 70 ml of light petroleum (B.P. 40–60°C.), or of petroleum benzin (B.P. 30–75°C.), and pour the solution gradually thru the column. Take care not to disturb the upper surface of the Al_2O_3 in adding the solution; assist filtration by gentle suction applied to the

filter flask, adjusting the pressure in the flask so that the rate of filtration does not exceed about 2 drops per second of liquid leaving the tube. Keep the Al_2O_3 continuously covered with a layer of solvent as previously described. When the solution has nearly all passed into the oxide layer, wash the column with light petroleum or petroleum benzin, passing in all about 150 ml of this thru the column. The tube should be nearly filled at each addition of the washing solvent, and the layer above the Al_2O_3 should be allowed to reduce to about 2 cm. in depth before the next addition of solvent is made.

Transfer the contents of the filter flask thru a filter to a weighed flask, rinsing and washing the filter and the flask into the weighed flask. Evaporate the solvent in the usual manner by distillation from a hot water bath, dry the residue in an oven at 100°C . for 10 minutes, cool in a desiccator, and weigh. Repeat the drying until the weight is constant to 1 mg.

Divide the weight of hydrocarbon obtained by the weight of oil originally taken and multiply by 100 to give the percentage.

DISCUSSION

This method has been used extensively in this laboratory for the separation of squalene from olive oil (4), and for the detection and determination of mineral oil in fatty oils that do not normally yield appreciable amounts of hydrocarbon when pure. Such oils include linseed oil, teaseed oil, and rapeseed oil; the method is also suitable for examining lanolin and sperm oil and certain sulphonated oils used in the leather industry. As is shown by the data recorded below, mineral oils have been found in the past few years to occur to varying small extents in the above oils.

TABLE 1.—*Recovery of hydrocarbons from pure fatty oils*

MATERIAL EXAMINED	UNSATURATED MATTER (S.P.A. METHOD)	HYDROCARBON SEPARATED
	<i>per cent</i>	<i>per cent</i>
(a) Oil prepared in laboratory:		
Linseed oil no. 1	1.18	0.05
Linseed oil no. 2	1.25	0.07
Linseed oil no. 3	1.12	0.04
(b) Oils believed to be free from mineral oil:		
Sperm oil no. 1	42.9	0.10
Sperm oil no. 2	43.4	0.07
Linseed oil no. 4	1.16	0.05
Linseed oil no. 5	1.14	0.03
Teaseed oil no. 1	0.89	0.02
Teaseed oil no. 2	0.81	0.03
Rapeseed oil no. 1	1.07	0.06
Rapeseed oil no. 2	1.16	0.04

The hydrocarbon extracts from the oils known and believed to be uncontaminated with mineral oil were usually yellow in fluorescence when examined in ultra-violet radiation. In daylight they appeared colourless.

All the extracts from the oils of Table 2, and those from the last two oils of Table 3 were also colourless in daylight but showed a pale-blue fluorescence in ultra-violet radiation.

It is interesting to note that if the figures for the hydrocarbon content of the linseed oils are plotted against the respective percentages of un-

TABLE 2.—*Recovery of hydrocarbons from commercial fatty oils*

MATERIAL EXAMINED	UNSATONIFIABLE MATTER (S.P.A. METHOD)	HYDROCARBON SEPARATED
	<i>per cent</i>	<i>per cent</i>
Oils imported to Great Britain 1947/8		
Linseed oil no. 6	1.59	0.40
Linseed oil no. 7	1.29	0.19
Linseed oil no. 8	1.39	0.26
Linseed oil no. 9	1.81	0.58
Linseed oil no. 10	2.39	1.23
Linseed oil no. 11	1.95	0.83
Linseed oil no. 12	2.23	0.98
Linseed oil no. 12	3.17	2.01
Teaseed oil no. 3	2.28	1.34
Teaseed oil no. 4	1.37	0.52
Teaseed oil no. 5	2.77	1.79
Teaseed oil no. 6	2.46	1.70
Rapeseed oil no. 3	1.48	0.33

TABLE 3.—*Recovery of hydrocarbons from known mixtures*

MATERIAL EXAMINED	UNSATONIFIABLE MATTER (S.P.A. METHOD)	HYDROCARBON SEPARATED
	<i>per cent</i>	<i>per cent</i>
Linseed oil no. 1	1.18	0.05
Same oil +0.2 per cent of mineral oil	1.40	0.23
Same oil +0.5 per cent of mineral oil	1.67	0.51

saponifiable matter in a graph, the points lie close to a straight line of unit slope. From this and the other facts adduced, it is inferred that the percentage of hydrocarbon extracted is less than 0.1 per cent higher than the percentage of adventitious mineral oil present in a sample.

SUMMARY

A chromatographic method is described for the recovery of hydrocarbons from the unsaponifiable matter of fatty oils. Results are given for the application of the method to pure and contaminated oils; it is shown to yield figures within 0.1 per cent of the amount of mineral oil present in samples of linseed, teaseed, and rapeseed oil. The method is of

general application to oils which do not yield hydrocarbons in a pure state.

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STUDIES ON COAL-TAR COLORS

FD&C YELLOW NO. 6 AND C.I. NO. 26

By CHARLES STEIN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

FD&C Yellow No. 6 (Sunset Yellow), the disodium salt of 1-(4 sulfo-phenol)-2-naphthol-6-sulfonic acid, is listed as certifiable for use in foods, drugs, and cosmetics under the authority of the Federal Food, Drug, and Cosmetic Act of 1938 (1). This study was undertaken in order to make available a sample of pure FD&C Yellow No. 6, which is to be used to check the validity and accuracy of the procedures ordinarily employed in the examination of this color.

The sodium salt of 1-phenyl-azo-2-naphthol-6-sulfonic acid (C.I. No. 26) (2) is a possible contaminant in commercial samples of FD&C Yellow No. 6. Since the regulations specify the amount of subsidiary dye allowable in certifiable samples of FD&C Yellow No. 6, a sample of C.I. No. 26 was prepared to aid in the development of a method for subsidiary dyes in FD&C Yellow No. 6.

The purified materials were also employed in our investigation of the spectrophotometric characteristics of coal-tar colors. Solutions of the colors were tested spectrophotometrically to determine the location of the absorption peaks, the conformity of the colors to Beer's law, and the effect of pH on the absorption spectra as an aid in identifying the color.

EXPERIMENTAL

Schaffer's acid (2-naphthol-6-sulfonic acid) was prepared according to the procedure of Engel and Hutchison (3). Commercial β -naphthol (100 gms.) was sulfonated with 100 ml. of 100% sulfuric acid at 85° for 75 minutes. The reaction mixture was poured into 500 gm. of ice and water.

The resulting solution was filtered and the Schaffer's acid precipitated by saturating the cold solution with dry hydrogen chloride gas. The precipitated material was filtered on a fritted glass Büchner, washed with cold concd. hydrochloric acid, then dissolved in water and again precipitated with hydrogen chloride gas. This procedure was repeated until the precipitated material gave a negative test for sulfuric acid. The product was then placed in a desiccator over solid potassium hydroxide until free of hydrogen chloride. A small amount of the chloride-free material was dried in a desiccator over sulfuric acid to form the monohydrate. This melted at 129°, as stated by Engel and Hutchison (3).

The bulk of the Schaffer acid was not dried. Its moisture content was determined before use by drying a sample at 135° overnight. Allowance for the moisture content was made in weighing portions of the material for use.

Sulfanilic acid was purified by repeated recrystallization of commercial material from water.

Aniline was purified by distillation.

PREPARATION OF FD&C YELLOW NO. 6

Sulfanilic acid, 8.7 gms. (0.05 mole), was dissolved in 100 ml. of hot water containing 2.65 gm. of sodium carbonate. After cooling to 20°C, 3.7 gm. of sodium nitrite was added, and the solution poured into a beaker containing 50 gm. of ice and 10 ml. of concd. hydrochloric acid. The temperature of the solution was kept below 5°C for 15 minutes. An excess of urea was then added to destroy the excess nitrous acid.

The suspension of the diazonium salt was poured into a cold (5°C) solution of 11.2 gm. (0.05 mole) of Schaffer's acid and 6.0 gm. of sodium hydroxide dissolved in 250 ml. of water. After stirring for one hour, the solution was removed from the ice bath and allowed to come to room temperature.

The acidity of the reaction mixture was adjusted to a pH of 2, the solution was boiled for 4–5 minutes to destroy carbonates and bicarbonates, and then evaporated to dryness on the steam bath. The solid material was dissolved in 200 ml. of water, and the solution heated to boiling. The dye was precipitated by the addition of 1000 ml. of alcohol, filtered with suction, and washed with a 5:1 alcohol-water mixture. The color was reprecipitated twice using 100 ml. of water and 500 ml. of alcohol each time. After a preliminary drying on the steam bath, the purified color was dried at 135°C. Yield: 19.2 grams.

Analytical data on the purified FD&C Yellow No. 6 are shown in Table 1.

PREPARATION OF C.I. NO. 26

Aniline, 6.52 gm., (0.07 mole) was agitated with 25 ml of hot water, and 17.5 ml of concd. hydrochloric acid was added in a thin stream. The

TABLE 1.—Analytical data*—Purified FD&C Yellow No. 6

DETERMINATION	FOUND	CALCULATED
Nitrogen, per cent	6.1	6.19
Sulfur, per cent	14.1	14.2
Sodium (from sulfated ash) per cent	9.9	10.2
Titration (ml. 0.1 N TiCl_3 per gram)		
a. Sodium citrate as buffer	88.45	88.45
b. Sodium bitartrate as buffer	88.2	
Inorganic sulfates, chlorides, and carbonates	Nil	—

* All samples were dried in an Abderhalden drier at 135°C. and 6 mm pressure.

solution was allowed to cool to 40° and sufficient ice was added to lower the temperature to 0°, leaving a slight excess of ice. A solution of 4.9 gm. of sodium nitrite in 25 ml. of water was added rapidly with stirring. After several minutes, the excess nitrous acid was destroyed with urea.

The solution of benzene diazonium chloride was added slowly, with stirring, to a cold 5°C solution of 15.7 gm. (0.07 mole) of Schaffer's acid and 10.5 gm. of sodium hydroxide in 300 ml. of water. After 1 hour the solution was removed from the ice bath and permitted to warm to room temperature.

The dye was then isolated and purified in the same way as the FD&C Yellow No. 6. The yield of purified product was low, 8 gm., but spectrophotometric analysis of the combined filtrates revealed the presence of 13.3 gm. of the dye.

Analytical data on the purified material are shown in Table 2.

TABLE 2.—Analytical data—Purified C.I. No. 26*

DETERMINATION	FOUND	CALCULATED
Nitrogen, per cent	7.8	8.0
Sulfur, per cent	9.1	9.15
Titration (ml 0.1N TiCl_3 per gram)	112.5	114.2
Inorganic sulfates, chlorides and carbonates	Nil	—

* Sodium salt of 1-(phenylazo)-2-naphthol-6-sulfonic acid.

DISCUSSION

The analytical data on the purified samples indicate a purity of at least 99 per cent for the FD&C Yellow No. 6 and over 98 per cent for the C. I. No. 26. Both samples appear to be sufficiently pure to serve as standards.

The data show that the titanium trichloride titration ordinarily used for the determination of the "pure dye" content of samples of these colors is accurate to within ± 1 per cent. Essentially the same result is obtained in the titration of FD&C Yellow No. 6 with either sodium citrate or sodium bitartrate as the buffer.

When the purified FD&C Yellow No. 6 was analyzed by the A.O.A.C. procedure for lower sulfonated (4) subsidiary dyes in FD&C Yellow No. 6, no subsidiary dye was found. When known amounts of C.I. No. 26 were added to the purified FD&C Yellow No. 6, 98 per cent of the added dye was recovered by this procedure. Three commercial samples of FD&C Yellow No. 6 were examined for subsidiary dyes by the A.O.A.C. procedure and found to contain 0.2–0.9 per cent subsidiary dye; however, the spectrophotometric curve of the extracted dye appeared to correspond more closely to that of D&C Orange No. 4 than to that of C.I. No. 26.

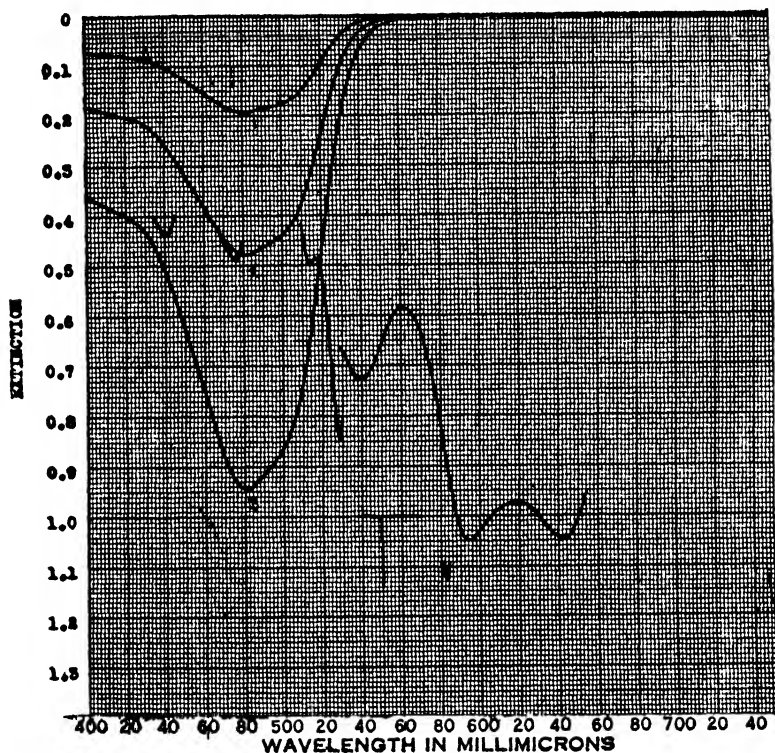


FIG. 1.—FD&C Yellow No. 6

Solvent: 0.02 N $NH_4C_2H_3O_2$

Concentration:

Curve 1— 3.48 mg. per liter

Curve 2— 8.70 mg. per liter

Curve 3—17.41 mg. per liter

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm.

(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$)

B = Corning Didymium Glass 592, 4.02 mm.

(Absorption peak at 583.7 $m\mu$)

C = Signal Lunar White Glass H-6946236.

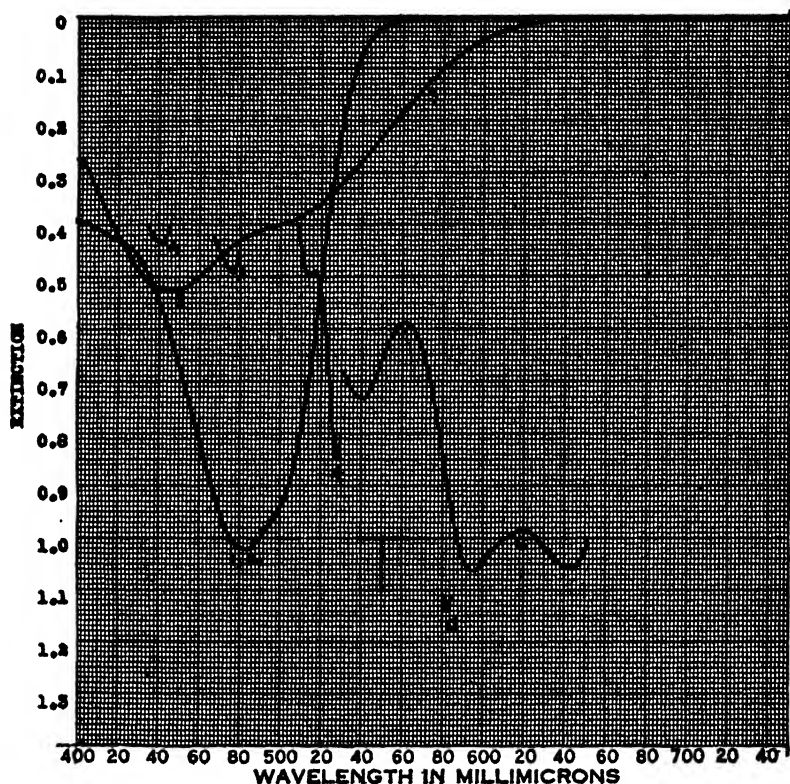


FIG. 2.—FD&C Yellow No. 6

Concentration: 1850 mg. per liter

Solvent:

Curve 1—0.02 $N \text{ NH}_4\text{C}_2\text{H}_3\text{O}_2$ Curve 2—0.1 $N \text{ HCl}$ Curve 3—0.1 $N \text{ NaOH}$

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm.

(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$)

B = Corning Didymium Glass 592, 4.02 mm.

(Absorption peak at 583.7 $m\mu$)

C = Signal Lunar White Glass H-6946236.

SPECTROPHOTOMETRIC DATA

All spectrophotometric measurements were made with a General Electric Recording Spectrophotometer equipped with an automatic slit adjustment for an 8 $m\mu$ wave length band.

The solutions for spectrophotometric analysis were prepared by dissolving a weighed portion of dye in water and diluting to exactly 1000 ml. To aliquots of this solution the appropriate buffer was added and the solution diluted to a definite volume. All solutions were made to volume

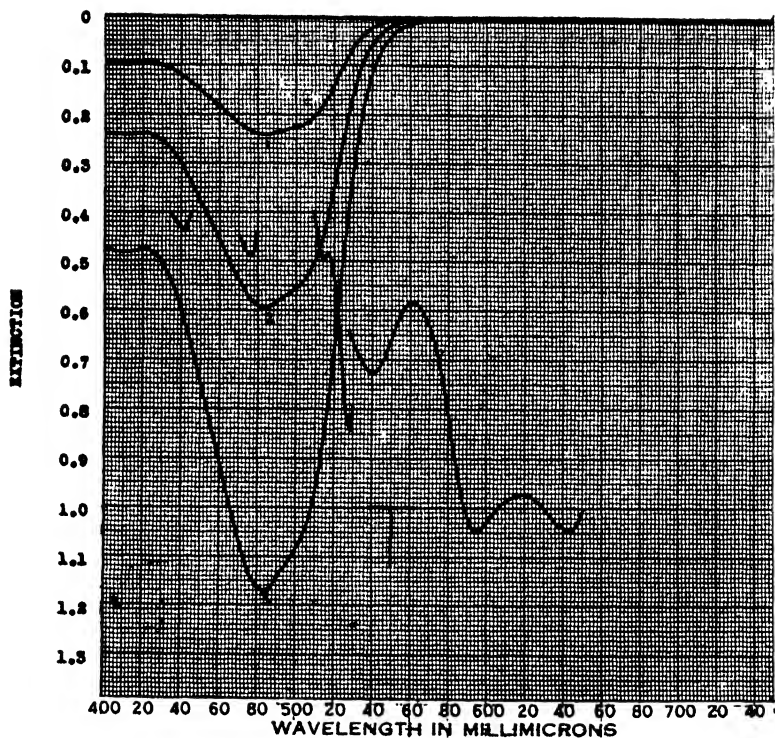


FIG. 3.—C.I. No. 26

Solvent: 0.02 N $NH_4C_2H_3O_2$

Concentration:

Curve 1— 3.97 mg. per liter

Curve 2— 9.94 mg. per liter

Curve 3—19.87 mg. per liter

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm.

(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$)

B = Corning Didymium Glass 592, 4.02 mm.

(Absorption peak at 583.7 $m\mu$)

C = Signal Lunar White Glass H-6946236.

at the temperature ($25 \pm 5^\circ C$) of the room in which the optical measurements were made.

Typical sets of curves for neutral solutions of FD&C Yellow No. 6 are shown in Figure 1 and for neutral, acid, and alkaline solutions of the color in Figure 2. Curves for solutions of C.I. No. 26 are shown in Figures 3 and 4.

The curve for neutral and acid solutions of FD&C Yellow No. 6 are identical with a peak at $482 \pm 2 m\mu$ and a shoulder at about $420 m\mu$. In strongly alkaline solution, the peak is shifted to $445 m\mu$ with a broad shoulder at about $500 m\mu$.

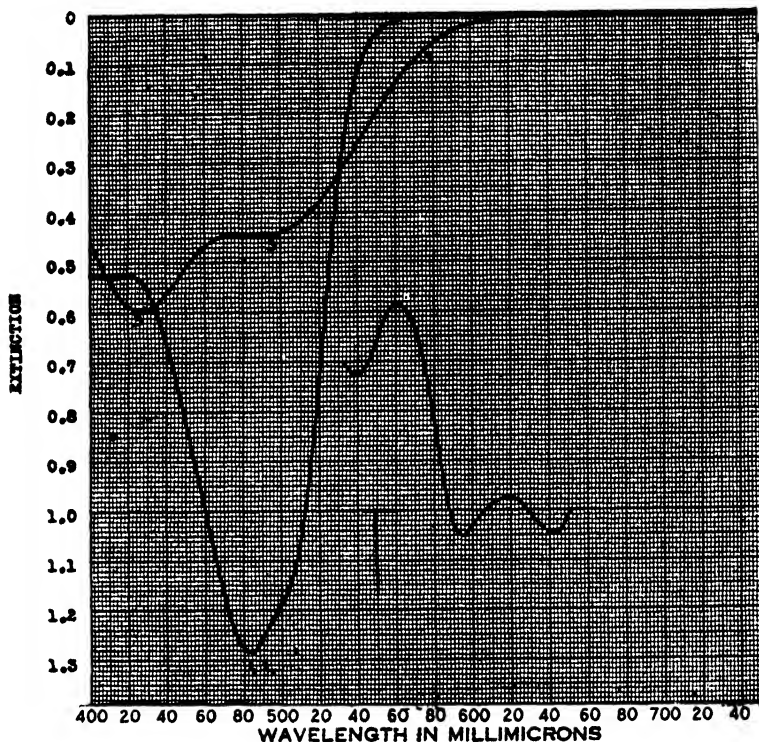


FIG. 4.—C.I. No. 26

Concentration: 21.77 mg. per liter

Solvent:

Curve 1—0.02 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$

Curve 2—0.1 N HCl

Curve 3—0.1 N NaOH

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm.

(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$)

B = (Corning Didymium Glass 592, 4.02 mm.

(Absorption peak at 583.7 $m\mu$)

C = Signal Lunar White Glass H-6946236.

Neutral solutions of FD&C Yellow No. 6 containing 4 to 25 mg. of dye per liter conform to Beer's law at the absorption peak. In six sets of three determinations each, the average extinction per milligram per liter at 482 $m\mu$ was 0.0547 with an average deviation of 0.34 per cent and a maximum deviation of 0.7 per cent.

The curves obtained on neutral, acid, and basic solutions of FD&C Yellow No. 6, which had aged for 24 hours, were identical with those of freshly prepared solutions.

The curves for neutral and acid solutions of C.I. No. 26 are identical

with a major peak at 484 ± 2 m μ and a secondary peak at 410 ± 2 m μ . In strongly basic solution, the dye gives peaks at 428 and 490 m μ .

Neutral solutions of C.I. No. 26, containing 4 to 20 mg. of color per liter, obey Beer's law to within ± 1 per cent. The average extinction per milligram per liter at 484 m μ was found to be 0.0597. Neutral, acid and basic solutions of the color are stable for at least 24 hours.

C. I. No. 26 can be differentiated spectrophotometrically from FD&C Yellow No. 6 by its secondary peak at 410 m μ in neutral solution, or more readily by its curve in alkaline solution.

Since both of the colors covered in this report obey Beer's law, each dye can be determined conveniently by spectrophotometric examination if other colored substances are absent.

Spectrophotometric curves are now drawn routinely for all samples of FD&C Yellow No. 6 submitted for certification. For ten samples selected at random from the files the average deviation between the spectrophotometric and titration values for "pure dye" was 0.9 per cent and the maximum deviation, 2.2 per cent.

SUMMARY

Samples of FD&C Yellow No. 6 and C. I. No. 26 sufficiently pure to serve as analytical standards have been prepared.

Each of these dyes can be accurately titrated with titanium trichloride.

The A.O.A.C. procedure for subsidiary dyes in FD&C Yellow No. 6 will determine C.I. No. 26 practically quantitatively. The three commercial samples of FD&C Yellow No. 6 examined for subsidiary dyes did not contain appreciable amounts of C.I. No. 26.

Spectrophotometric data on solutions of the two colors is presented. The curves of the two dyes are quite similar, with a major absorption peak at 480-485 m μ , but the two colors can be differentiated readily spectrophotometrically.

Neutral solutions of both the dyes follow Beer's law; hence, they may be determined spectrophotometrically if interfering substances are absent.

The spectrophotometric and titration values for "pure dye" content of most commercial samples of FD&C Yellow No. 6 agree to within ± 1 per cent.

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ACKNOWLEDGMENT

The author wishes to thank Mr. M. Dolinsky for making the spectrophotometric measurements.

VOLUMETRIC DETERMINATION OF CHLORINE
AND BROMINE IN ORGANIC COMPOUNDS

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The work done in this laboratory requires the determination of halogens in a number of coal-tar colors, intermediates, and other organic compounds. In the compounds analyzed, chlorine, bromine, and iodine occur alone and in various combinations. Simple, accurate, volumetric methods for iodine and bromine in such compounds are available (1, 2). Several volumetric methods for chlorine in organic compounds are described in the literature, but none of these methods is very satisfactory when considerable accuracy is required. For that reason, chlorine is usually determined gravimetrically. When a large number of determinations are to be made, an accurate volumetric method for chlorine would be a decided advantage.

Sendroy (4) has described a titrimetric method for inorganic halides based on the reaction of the halide ion with solid silver iodate which is simple, convenient, and accurate. This paper describes a method developed in this laboratory for the determination of chlorine and bromine in organic compounds using Sendroy's titration for the final determination.

METHOD

REAGENTS

Silver iodate.—Suitable for the determination of chloride. (Obtainable from Merck and Company, Rahway, New Jersey. See reference (4) for method of preparation.)

Sodium nitrite, 10%.

Sulfuric acid, 6 N.

Sulfamic acid, 10%.

Standard sodium thiosulfate, 0.05–0.1 N.—Standardize against KIO_3 .

PROCEDURE

Accurately weigh a sample which contains at least 15 mg of Cl or 30 mg of Br. Oxidize the sample and absorb the evolved halogen in a mixture of 15 ml of 1% hydrazine sulfate plus 5 ml of 10% NaOH as directed in the official A.O.A.C. procedure for bromine in halogenated fluorescein dyes (3).

Transfer the absorbing soln to a 200 ml beaker and wash the absorption flask with two 5–10 ml portions of H_2O . Complete the washing with 5 ml of the nitrite soln and 10 ml of 6 N H_2SO_4 , and add these reagents to the beaker. Thoroughly mix the resulting soln and allow to stand for at least 2 min. Wash down the sides of the beaker with 10 ml of the sulfamic acid soln and stir the mixture for 2 min. Add an excess (0.4–0.8 gram) of solid AgIO_3 , and mix vigorously for at least 2 min. Transfer the mixture to a 100 ml volumetric flask, cool to room temp., and make to volume with

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water. Mix thoroly and filter thru a dry fluted filter. Discard the first few ml of filtrate.

Dilute an aliquot of the filtrate to ca 100 ml with water, add 2 g of KI and titrate the liberated I with $\text{Na}_2\text{S}_2\text{O}_3$, using starch soln as the indicator.

$$\begin{aligned} 1 \text{ ml } 0.1 \text{ N } \text{Na}_2\text{S}_2\text{O}_3 &= 0.591 \text{ mg Cl} \\ &= 1.337 \text{ mg Br} \end{aligned}$$

If the sample contains both Br and Cl, dilute the soln to exactly 100 ml before the addition of silver iodate. Neutralize an aliquot of this soln (use at least half the soln) with 30 per cent NaOH and determine Br by the official A.O.A.C. procedure (3). To the remaining soln, add solid AgIO_3 , shake vigorously for at least 2 min., filter, and titrate an aliquot of the filtrate as directed above to obtain the total halide content (in mols).

The bromine and chlorine content of the sample is calculated from the equations:

$$\text{Br (mg)} = T_1 \times N_1 \times 39.96 \times \frac{100}{A}$$

$$\text{Cl (mg)} = [T_2 \times N_2 \times \frac{100}{B} - (3 \times T_1 \times N_1 \times \frac{100}{A})] \times 5.91$$

where

T_1 = titration for Br determination

T_2 = titration for total halide determination

N_1 = normality of $\text{Na}_2\text{S}_2\text{O}_3$ used in T_1

N_2 = normality of $\text{Na}_2\text{S}_2\text{O}_3$ used in T_2

A = aliquot used for the Br determination

B = aliquot used for the total halide determination.

DISCUSSION

The chromic-sulfuric acid digestion employed in this study is a simple, convenient method for the decomposition of a large number of compounds. One advantage of this digestion is that iodine is oxidized to iodate and is not carried into the absorbing solution with the bromine and chlorine. Although other methods of destroying the organic material have not been investigated, it appears probable that the alkaline hydrazine absorbent could be used with several other decomposition procedures described in the literature.

Sendroy (4) has discussed in detail the principles of his method. The accuracy depends somewhat on the amount of halide present but, for concentrations above a minimum of about 5 millimoles per liter, the results are accurate to within a few parts per thousand. The final titration is simple and precise and the end point is sharp and permanent.

Sendroy recommends the use of dilute phosphoric acid (0.085 mole) in the chloride determination, but notes that the acidity is not critical unless iodide is present. The reaction between hydrazine and nitrite does

not appear to be complete unless the pH of the solution is low. In our experiments, essentially the same results were obtained whether the determination was carried out in dilute (0.06 *N*) sulfuric acid or in phosphoric acid. (See Table 1.)

TABLE 1.—*Recoveries of chloride and bromide*

HALOGEN	AMOUNT	RECOVERY IN PRESENCE OF:		
		H ₃ PO ₄	H ₂ SO ₄	ABSORPTION SOLUTION
	mg	per cent	per cent	per cent
Chloride	15.3	100.0	100.5	100.3
Chloride	30.6	100.0	100.3	—
Chloride	61.2	99.6	99.6	—
Bromide	16.8	102.1	101.4	—
Bromide	33.6	100.2	100.3	99.9
Bromide	67.2	99.8	99.6	—

The effectiveness of the procedure used to remove the excess hydrazine was checked by the addition of the specified reagents to 25 ml. of the mixed absorbing solution. The final solution neither reduced iodate nor oxidized iodide; an aliquot of standard potassium iodate solution liberated the same amount of iodine from iodide in the presence of this mixture as an equal aliquot did in the presence of dilute sulfuric acid. The results obtained, when known amounts of chloride and bromide were added to the alkaline hydrazine solution and the mixture analyzed by the method described, are shown in Table 1. These results show that the reagents used do not interfere in the determination.

The only difficulties encountered in numerous determinations of chloride and bromide by this method have been occasional low results obtained in early experiments. These were found to be due to incomplete reaction between the halide and the silver iodate. No difficulty from this source will arise if the mixing is carried out as specified in the directions.

Typical results obtained in the analysis of organic halogen compounds by the proposed method are shown in Table 2. The average deviation from the calculated value for these compounds is 0.5 per cent and the greatest deviation 1.0 per cent.

In calculating the results shown in Table 2 no correction has been made for the difference in recovery at different halide concentrations. The precision of the final titration might justify such a correction, but variations introduced in steps prior to the titration are probably greater than this correction. For the same reason no correction has been made for the volume (about 0.1 ml.) occupied by the solid material.

In the determination of both bromine and chlorine, bromine is deter-

TABLE 2.—*Chlorine or bromine in organic compounds*

COMPOUNDS	HALOGEN	
	FOUND	CALCULATED
	<i>per cent</i>	<i>per cent</i>
<i>o</i> -Chlorobenzoic acid (N.B.S. standard sample No. 144)	22.6	22.65
	22.7	
4-Bromoacetanilide	37.4	37.34
	37.1	
4-Chlororesorcinol	24.7	24.54
	24.6	
	24.6	
2,5-Dichloracetanilide	34.5	34.76
	34.6	
	34.6	
Tetrabomphthalic anhydride	68.4	68.9
	68.2	
Tetrachlorophthalic anhydride	49.4	49.7
	49.2	
	49.6	
Tetrabromfluorescein diacetate	43.6	43.68
	43.8	
Tetrachlorfluorescein diacetate	25.7	25.60
	25.7	

TABLE 3.—*Mixtures of bromine and chlorine*

MIXTURE NO.	BROMINE*		CHLORINE*	
	ADDED	FOUND	ADDED	FOUND
	mg	mg	mg	mg
1	33.6	33.5	15.2	15.0
2	83.9	83.3	15.2	15.3
3	83.9	83.2	15.2	15.4
4	67.1	66.8	30.3	30.4
5	67.1	66.7	30.3	30.5
6	33.6	33.3	151.6	151.8
7	33.6	33.2	151.8	150.8

* Added as KBr and NaCl, respectively.

mined directly and chlorine by difference. Both of these determinations must be made quite accurately if the results are to be satisfactory. Typical results obtained in the analysis of mixtures of chloride and bromide by the method proposed in this paper are shown in Table 3. The results are not quite as accurate as those usually obtained when the total halide content is determined gravimetrically, but are sufficiently accurate for most purposes.

SUMMARY

A simple, rapid, volumetric method for the determination of bromine and chlorine in organic compounds has been presented.

The compound is oxidized with chromic and sulfuric acids and the halogen evolved is absorbed in alkaline hydrazine. The excess hydrazine is destroyed with nitrite in acid solution and the excess nitrite removed with sulfamic acid. The halide is then determined by adding solid silver iodate and determining the iodate ion liberated as proposed by Sendroy.

If both bromine and chlorine are present, bromine is determined in a separate aliquot by the A.O.A.C. procedure and the chlorine content calculated by difference from the total halogen determined.

Typical results are given.

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THE DETERMINATION OF GAMMA-BENZENE HEXACHLORIDE IN INSECTICIDE PRODUCTS

By THOMAS H. HARRIS (Insecticide Division, Livestock Branch,
Production and Marketing Administration, U. S. Department
of Agriculture, Beltsville, Maryland)

Several independent methods for determining gamma-benzene hexachloride have recently been published (1-9). The conditions for separating the isomers of benzene hexachloride on a partition chromatographic column were worked out by Ramsey and Patterson (10), and this work furnished the basis for a quantitative method described by Aepli, Munter, and Gall (1). The method of Aepli and co-workers has been in use in this laboratory for some time and its accuracy and precision have been confirmed. The method is not entirely suitable, however, for routine work where the analysis of large numbers of samples are required. The purpose of the work reported here was to simplify this method and retain the accuracy and precision of the original method.

The procedure of Aepli and co-workers requires the use of 100 g of silicic acid and the collection of 30–40 ten ml fractions after 100 ml of solvent have passed through the column. The gamma isomer is located by evaporation of the fractions and identification by its characteristic crystalline appearance.

It was apparent after some study of this method that the number of fractions collected could be reduced if some means could be found of visually locating or marking the position of the gamma isomer band as it moved down the column. For this purpose a number of oil-soluble coal-tar colors appeared promising because of their solubility in, and distribution between, the two immiscible solvents, nitromethane and normal hexane. One of these dyes, D and C Violet No. 2 (1-hydroxy-4-p-toluinanthraquinone) when added to a solution of gamma-benzene hexachloride to be chromatographed, was observed to move down the column just ahead of the gamma isomer, and largely with the alpha isomer which precedes the gamma isomer. With this dye present and serving as a marker for the position of the gamma isomer the collection of fractions could be delayed until all but the last trace of the dye had left the column.

A modified partition chromatographic procedure, using the above mentioned dye as a marker for the front of the gamma isomer band, is described and has been found useful in this laboratory for routine work.

APPARATUS

(1) *Partition column*.—The column and reduction valve are shown in Figure 1. The column is 72 cm long and 2.5 cm in diameter. A fritted glass disc is sealed in place and 5 cm below the disc is attached a ball and socket joint No. 18/7. The lower end of the female part of the joint is constricted to a diameter of about 5 mm. The column shown was constructed of standard wall Pyrex glass tubing by the Scientific Glass Apparatus Company.¹ The oxygen-type reduction valve was obtained from the Southern Oxygen Company.¹ Pressure is supplied from a laboratory air pressure line.

(2) *Solvent evaporator*. The solvent evaporator is shown in Figure 2. The fractions are evaporated to dryness under reduced pressure at 60°C¹, with the aid of a water pump. The solvent is recovered in a trap consisting of a Kjeldahl flask immersed in a mixture of salt and ice.

(3) *Erlenmeyer flask*.—125 ml.

(4) *Graduated cylinders*. Two 10 ml.

(5) *Pipets*.—Ten ml volumetric and 5 ml serological.

(6) *Volumetric flask*.—50 ml glass-stoppered.

REAGENTS

(1) *Normal hexane*.—Phillip's Petroleum Company¹ commercial grade.

(2) *Nitromethane*.—Commercial Solvents Company¹ Redistilled before use.

(3) *Silicic acid*.—Mallinckrodt's¹ reagent grade

(4) *Dye solution*.—Dissolve 25 mg of D and C violet No. 2² (1-hydroxy-4-p-

¹ The mention of these products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over others of a similar nature not mentioned.

² Available from Pylam Products, 799 Greenwich Street, New York, 14, N. Y.

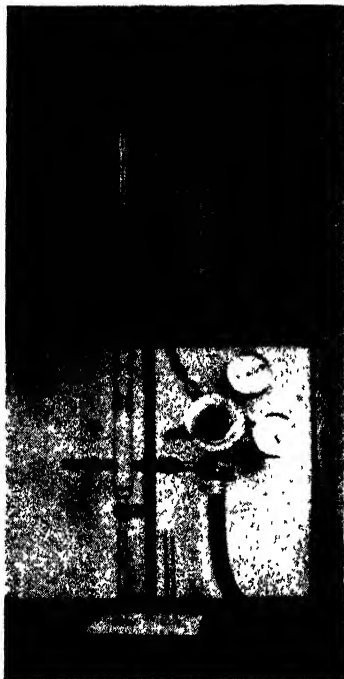


FIG. 1.—Partition column.



FIG. 2.—Solvent evaporator.

(toluino-anthraquinone) in 50 ml of mobile solvent and store in a glass-stoppered bottle.

(5) *Mobile solvent*.—This is a saturated solution of nitromethane in normal hexane. Shake vigorously 2 liters of normal hexane with an excess of nitromethane in a glass-stoppered bottle. Decant the mobile solvent from the nitromethane as needed.

PREPARATION OF SAMPLE

(a) *Powders with more than 10% gamma-benzene hexachloride*.—Weigh a quantity of finely powdered sample sufficient to provide 150–200 mg of gamma-benzene hexachloride and transfer to a 125 ml glass-stoppered Erlenmeyer flask. Add 25 ml of mobile solvent, heat just to boiling, and allow to cool to room temperature with occasional shaking. Then decant the extract thru a small conical filter paper into a 50 ml glass-stoppered volumetric flask containing 1 ml of dye solution. Make a second hot extraction with 5 ml of mobile solvent. Rinse the residue 4–5 times using 5 ml portions of mobile solvent at room temperature. Finally dilute to volume with mobile solvent and mix.

(b) *Powders containing less than 10% gamma-benzene hexachloride*.—Transfer the weighed sample to a Soxhlet extractor and extract overnight with ethyl ether. Evaporate most of the ether on a steam bath and, finally, the remainder at room temperature under reduced pressure. Extract the gamma-benzene hexachloride from the residue with mobile solvent as directed above. (Samples containing less than 10% gamma-benzene hexachloride are difficult to extract directly with a minimum of mobile solvent owing to the bulk of siliceous filler generally present.)

(c) *Emulsion concentrates*.—Weigh a sample and transfer to 125 ml glass-

stoppered Erlenmeyer flask. Add 1–2 g of silicic acid to retain the water and extract with mobile solvent as directed above.

(d) *Solutions in petroleum distillate*.—Weigh a sample and dilute to 50 ml in a volumetric flask with mobile solvent.

PREPARATION OF COLUMN

Weigh 50 g (± 5 g) of silicic acid and transfer to a Waring blender. Add 150 ml of mobile solvent and, with mixing, add 27 ml of nitromethane. After mixing for 15 seconds in the blender, pour the mixture quickly into the column thru a glass funnel. Wash down the sides of the column with a few ml of mobile solvent and then apply 2–3 pounds pressure until all of the solvent is just forced into the column of silicic acid. Release the pressure by cautiously removing the rubber stopper at the top of the column so as not to disturb the silicic acid.

OPERATION OF THE COLUMN

Pipet out 10 ml of the solution and allow to flow slowly down the inside of the column without disturbing the surface of the silicic acid. Wash down the side of the column with 1–2 ml of mobile solvent and force the solution into the silicic acid by applying 2–3 pounds pressure. Release the pressure and slowly add 150 ml of mobile solvent, down the inside wall of the column. Apply 2–3 pounds pressure, which forces the solvent thru the column at a rate of about 3–4 ml per minute. Just before the last trace of dye leaves the column, begin to collect 5 ml fractions, alternately using two 10 ml graduated cylinders. Transfer each fraction to a 125 ml Erlenmeyer flask and evaporate to dryness, using the solvent evaporator. The fractions should be evaporated without boiling. If boiling occurs, raise the flasks momentarily from the water bath.

The appearance of the gamma isomer will be recognized upon evaporation by its tendency to cover the bottom of the flask as a white residual film. When the first residue of gamma isomer is recognized begin to collect 10 ml fractions until all of the gamma isomer is obtained, usually about 8 fractions. Dissolve the residue in each flask with normal hexane and transfer to a weighed 125 ml Erlenmeyer flask by means of a 5 ml serological pipet. Evaporate the solvent by using the solvent evaporator. Evacuate the flask¹ for about 5 minutes at room temperature with a vacuum pump. Release the vacuum, wipe the flask with a clean, moist towel and allow to stand for 5 minutes. Weigh and calculate the percentage of gamma-benzene hexachloride in the original sample.

Prepare the column for the next analysis as follows:

Extrude the silicic acid by applying gentle pressure. Clean the column with a long handled brush, wash with water and acetone, and dry by attaching to a vacuum line.

DISCUSSION

Following the procedure previously outlined, the data shown in Tables 1 and 2 were obtained. The accuracy and precision of the results shown may be expected in this method after one becomes experienced with the technique of preparing and operating the column.

The present method has been applied to a number of insecticidal powders and none of the other ingredients present have been found to cause

¹ There appears to be little or no danger in evacuating a 125-ml Erlenmeyer flask. A larger size Erlenmeyer flask, however, is likely to collapse under vacuum.

any interference. The wetting agents generally used in the wettable-powder-type products appear to be only slightly soluble in the solvents employed.

DDT causes no interference and is easily separated from the gamma isomer on the column. The quantitative determination of DDT by this same procedure is at present being investigated in this laboratory. Monochloronaphthalene, dichloronaphthalene, and trichloronaphthalene pres-

TABLE 1.—*Determination of gamma-benzene hexachloride in the presence of the other isomers*

SOLUTION	BHC ISOMERS ADDED				GAMMA ISOMER RECOVERED
	ALPHA	BETA	DELTA	GAMMA	
	mg				mg
1	—	—	—	100	100.8
2	25	—	—	200	198.4
2	25	—	—	200	199.0
3	100	10	20	100	101.0
3	100	10	20	100	99.9
4	100	—	—	12.5	13.2
4	100	—	—	12.5	12.4
4	100	—	—	12.5	12.3

TABLE 2.—*Analysis of commercial insecticide products. Precision of method*

SAMPLE	PER CENT GAMMA-BENZENE HEXACHLORIDE	
	(1)	(2)
a.	2.89	2.92
b.	3.44	3.42
c.	5.18	5.20
d.	11.3	11.3
e.	13.9	14.1
f.	15.3	15.5
g.	36.4	36.5

ent in one product analyzed caused no interference in the determination of gamma-benzene hexachloride. Some difficulty has been experienced, however, in the analysis of certain liquid concentrates containing methyl naphthalenes.

Numerous advantages result from the use of the dye to locate or mark the position of the gamma isomer on the column. In reducing the quantity of silicic acid, from 100 g as recommended in the method of Aepli and coworkers, to 50 g in the present method the same sharp separation of the gamma isomer is achieved if 5 ml fractions instead of the 10 ml fractions are collected. In using the smaller quantity of silicic acid

there is a sufficient space in the column above the silicic acid to hold the necessary volume of solvent required for analysis, thus eliminating the need for a solvent reservoir as required in the Aepli method. The time required to chromatograph a solution is reduced from about three hours to approximately 45 minutes.

Furthermore, the simplified apparatus required in the present method makes it feasible to operate a battery of several columns simultaneously.

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The author wishes to thank Dr. G. Robert Clark of the Cosmetic Division, Food and Drug Administration, for supplying the coal-tar colors used in this study.

SUMMARY

A modified partition chromatographic method is presented for the determination of gamma-benzene hexachloride in insecticide products. A violet dye, D and C Violet No. 2 (1-hydroxy-4-p-toluidino anthraquinone) is added to the solution aliquot to be chromatographed and serves as a visible marker for the front of the colorless gamma-benzene hexachloride band as it moves down the column. The use of this dye as a marker results in a number of advantages, chief among which are fewer fractions collected, simplified apparatus, and more rapid analysis. Furthermore, the simultaneous operation of several adsorption columns is made feasible. DDT, chloronaphthalenes, and other ingredients often present in these products, offer no interference. The accuracy and precision of the method are satisfactory for the analysis of most commercial insecticide products containing gamma-benzene hexachloride.

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BOOK REVIEW ✓

Trace Elements in Food. By G. W. Monier-Williams. 511 pp. text, refs. and index. John Wiley and Sons, New York. Price \$6.00.

The scope of the book is best presented by quotation and paraphrase of the author's preface: "The term 'trace element' is usually applied . . . to those elements which, in extremely small amounts, play some part in the nutrition of plants or animals. It may equally well be applied to the larger number which have . . . a toxicological rather than nutritional interest, and the presence of which in food must, on public health grounds, be subject to close scrutiny and control." It is pointed out that, within limits, some are innocuous or even essential and the necessity of defining the upper limits, or tolerances, often confounds the food chemist. "He must therefore have . . . knowledge . . . of all the questions involved; the biochemistry, nutritional significance and toxicology . . .," also, of existing laws and regulations. The source of trace elements in foods, natural or adventitious, is considered and lastly, methods of analysis are outlined for each.

Trace elements are defined as those present in certain foods and in the human body in amounts up to .005 per cent (50 parts per million). Twenty-eight chapters are devoted, respectively, to copper, lead, zinc tin, arsenic, antimony, selenium, iron, nickel, cobalt, manganese, iodine, bromine, fluorine, boron, silicon, aluminum, silver, cadmium, chromium, bismuth, mercury, molybdenum, vanadium, titanium, indium, barium and strontium, and lithium and other metals. This listing of trace elements is stated to depend partly upon their relative importance as constituents of food and partly upon chemical relationships.

Each chapter comprises a discussion of the biological significance of the element; whether or not it is essential to plant or animal life; its toxicity, if any; the sources and amounts found in foods; legal limits if any; special topics; and, lastly, outlines of methods for its determination in foods. This discussion of methods comprises roughly about half of each chapter. Methods are usually given in outline, but some are presented in sufficient detail to serve as a "laboratory guide."

The book is profusely referenced (over 250 refs. in the copper chapter alone) and should be a source of much information for the toxicologist and food chemist.

P. A. CLIFFORD

REPORT ON INERT MATERIALS IN FERTILIZERS*

By K. G. CLARK,¹ *Associate Referee*, L. F. RADER, JR.,²
and H. R. WALLS³

A committee to study the problem of fillers and related materials in fertilizers was appointed by the Association of American Fertilizer Control Officials early in 1947.

This committee proposed that a nation-wide survey be conducted on the filler content of commercial fertilizer mixtures. To implement this proposal it appointed a subcommittee⁴ to develop methods which could be used in such a survey to indicate the filler content of fertilizers. At the same time the committee authorized a statement of policy which read in part as follows:

"... If the results of this survey warrant, the Association of Official Agricultural Chemists will be requested to appoint a referee to study this problem. Furthermore, it is the aim and desire of this committee to work in full cooperation with the Association of Official Agricultural Chemists and will present to them the results of our findings for their consideration."

From its studies the subcommittee devised methods for the determination of (a) calcium carbonate equivalent of carbonate carbon, (b) ash in acid-insoluble matter (acid-insoluble matter minus loss on ignition) and ash-free acid-insoluble organic matter (loss on ignition of acid-insoluble matter), and (c) ash-free water-insoluble organic matter. It submitted these methods to several State fertilizer control laboratories for evaluation as to their applicability in the proposed survey. Specially prepared mixtures also were made available to aid in the evaluation.

At this juncture the Association of Official Agricultural Chemists was requested to appoint, and appointed, an Associate Referee to study the proposed methods and others of possible use in the determination of filler.

This report presents the methods proposed by the above-mentioned sub-committee and the results obtained by the several collaborators in their evaluation tests.

METHODS

I. CARBONATE CARBON OR CALCIUM CARBONATE EQUIVALENT

APPARATUS

The apparatus consists of a Knorr alkalimeter (250-ml flask), the guard tube of which is filled with Ascarite (sodium hydroxide asbestos absorbent mixture) and the

* This report was presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11-13, 1948, and should have been printed with other reports on Fertilizers in the May (1949) *Journal*.

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condenser outlet connected with a CO_2 absorption train. The train consists of a Schwartz drying tube containing Ag_2SO_4 saturated H_2SO_4 (1+1) to a depth of ca $\frac{1}{2}$ -inch above the bend, a Bowen potash bulb or Schmitz drying tube containing the same soln, a second Schwartz tube filled with Anhydrone (anhydrous magnesium perchlorate), two Stetser and Norton absorption bulbs, the inlet two-thirds of which are filled with Ascarite and the outlet third with Anhydrone for absorbing the evolved CO_2 , a third Schwartz tube similarly filled with Ascarite and Anhydrone, a Drechsel gas washing bottle containing conc H_2SO_4 to a depth of 1–2 cm, a protecting trap, and an aspirating bottle or suction source.

DETERMINATION

Aspirate a slow stream of air thru the assembled apparatus until the two Stetser and Norton bulbs have reached a constant weight. Close off the train by turning the necessary stoppers, remove the dry alkalimeter flask, and place 5 g of fertilizer therein, replace the flask, fill the dropping funnel with 50 ml HCl (1+4). Slowly add the acid to the sample while aspirating. After the reaction has substantially subsided, gently heat the flask until the soln boils, and continue boiling for 2–3 min. Discontinue heating and continue aspirating the system for 20 min. Close off the system, remove the two Stetser and Norton bulbs, and hang in a balance case until cool enough to weigh (usually 20–30 min.). Before weighing, the bulbs should be wiped with a dry lint-free cloth using a standardized procedure. Weighings should be made against a tare, packed in the same manner as the absorption bulbs. The increase in weight of the absorption bulbs is due to CO_2 . Calculate and report the result as CaCO_3 equivalent.

II. ASH IN ACID-INSOLUBLE MATTER AND ASH-FREE ACID-INSOLUBLE ORGANIC MATTER

DETERMINATION

Weigh and place 5.0 g of fertilizer in a 250-ml beaker. Add 25 ml of hot H_2O (98–100°C.), swirl contents of beaker, decant and filter the liquid (11 cm Whatman No. 40, or equivalent rapid filtering paper), transfer residue in the beaker to the filter with a stream of hot H_2O from a wash bottle, and wash 4–5 times. Transfer the washed residue from the filter paper to the beaker by unfolding the paper and directing a stream of HCl (1+1) upon it from a wash bottle. Add HCl (1+1) to give a total volume of 100–125 ml. Cover the beaker with a watch-glass and boil for 15–20 min. Filter thru a tared Gooch crucible with an acid-washed asbestos mat and perforated porcelain plate to hold the mat in place. Wash the insoluble residue 6 times with hot H_2O , dry crucible 1 hr. at 125°C, cool in a desiccator, and weigh. The increase in weight represents the total acid-insoluble matter. Ignite crucible and contents 1 hr at 600°C. in a muffle furnace, cool, and reweigh. Report the loss on ignition as ash-free acid-insoluble organic matter and the net increase in weight of the crucible as ash in acid-insoluble matter.

III. ASH-FREE WATER-INSOLUBLE ORGANIC MATTER

REAGENTS

(a) *CaCl₂ soln, sp. gr. 1.40.* Prepare a saturated CaCl_2 soln by dissolving $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in hot water. The quantity of the salt required depends on its purity, but usually two pounds per liter of H_2O is sufficient to produce the desired soln, sp. gr. 1.40 at 25°C. Check the sp. gr. with an hydrometer, and if necessary heat the soln with an excess of the salt until a soln of the proper density is obtained. Decant the soln into a suitable bottle for storage. Add 4.0 ml conc HCl (sp. gr. 1.19) per liter of soln, and thoroly mix. Thoroly mix the stock soln each time before withdrawing a portion for use.

(b) *Ammonium carbonate.*

DETERMINATION

Measure 50 ml of the acidified stock soln into a 100-ml tall-form beaker. Place 5.0 g of the fertilizer in a dry beaker, add ca 0.05 g ammonium carbonate, and mix thoroly with a dry stirring rod. Transfer the dry mixture with the use of a camel's-hair brush to calcium chloride soln. Stir carefully but vigorously until sample is thoroly wet. (Proper grounding of the grinding mill during preparation of the sample has been found largely to overcome the difficulty of wetting some samples). Carefully wash and remove the stirring rod, and rinse down the sides of the beaker with a jet of the CaCl_2 soln from a wash bottle. Set the beaker aside for 10 to 30 minutes to permit the organic matter to collect on the surface of the soln. Remove the organic matter by skimming the surface of the liquid, using a tube connected to a source of vacuum thru a wide-mouth bottle (4-8 oz.) that traps the organic matter and any CaCl_2 soln carried with it. Care should be taken to remove as little CaCl_2 soln with the organic matter as possible. Rinse any organic matter remaining in the collecting tube into the bottle. Swab the sides of the beaker at the surface of the soln with a small wet piece of filter paper and rinse any adhering organic matter into the bottle. Transfer the collected organic matter to a previously tared Gooch crucible with asbestos mat, wash thoroly with hot water to remove all CaCl_2 . Drench the residue in the crucible once or twice with HCl (1+49) to destroy any adhering carbonate, wash with H_2O , dry 1 hr. at 125°C ., and weigh. Ignite 1 hr. at 600°C . and reweigh. Report the loss on ignition as ash-free water-insoluble organic matter.

COLLABORATORS

- (1) Bureau of Chemistry, California Department of Agriculture, Sacramento Calif.
- (2) Chemical Division, Florida Agricultural Department, Tallahassee, Fla.
- (3) Department of Chemistry, New Jersey Agricultural Experiment Station, New Brunswick, N. J.
- (4) Division of Fertilizer and Agricultural Lime, Bur. Plant Ind., Soils, and Agr. Eng., Beltsville, Md.

SAMPLES

The formulas of five mixtures which were submitted to collaborators for evaluation of the methods are given in Table 1. The principal sources of carbonate carbon in Sample 1 were Cal-Nitro and selectively calcined dolomite, whereas the principal source in the other four samples was dolomite. Three samples (1, 2, and 5) were formulated with a single source of insoluble organic material and the others (3 and 4) with two and three sources, respectively. Only Samples 1 and 3 were formulated without the use of sand as a make-weight material.

RESULTS OBTAINED BY COLLABORATORS

The results reported by the collaborators are presented in Tables 2 to 5, inclusive. Collaborator No. 3 was not equipped for the determination of carbonate carbon. Collaborator No. 2 compared the acid solubility in HCl (1+4) in addition to using the suggested concentration of 1+1. Collaborator No. 1 found it desirable to reduce the sample size from 5 g to 1 g in conducting the flotation separation of organic material. Collaborator No. 2 also varied the procedure for collecting the floated material by overflowing it from a small beaker.

TABLE 1.—*Formulas of mixtures submitted to collaborators*

SAMPLE NUMBER	1	2	3	4	5
GRADE	8-15-15	6-8-6	3-9-6	2-9-9	3-12-6
<i>Material</i>	<i>Pounds per ton</i>				
Nitrogen solution 2A, 40.6% N	—	—	—	—	142
Ammonium sulfate, 20.8% N	205	420	—	150	21
Ammonium nitrate, 32.5% N	100	100	—	—	—
Uramon, 42.0% N	—	—	100	—	—
Cal-Nitro, 20.5% N	260	—	—	—	—
Sodium nitrate, 16.0% N	—	—	—	95	—
Garbage tankage, 2.4% N	—	80	—	—	—
Cocoa shell meal, 2.4% N	100	—	—	—	—
Cotton seed meal, 5.7% N	—	—	300	100	—
Processed tankage, 8.5% N	—	—	—	60	—
Fish scrap, 6.1% N	—	—	—	50	—
Peanut hull meal, 1.5% N	—	—	100	—	—
Hyper-humus, 1.0% N	—	—	—	—	100
Ammo-Phos, 10.8% N, 47.0% P ₂ O ₅	280	—	—	—	—
Superphosphate, 20.0% P ₂ O ₅	—	800	900	900	1232
Double superphosphate, 46.0% P ₂ O ₅	420	—	—	—	—
Potassium chloride, 62.4% K ₂ O	515	195	95	80	205
Potassium sulfate, 48.0% K ₂ O	—	—	100	150	—
Sulfate of Potash-Mg, 27.0% K ₂ O	—	—	50	—	—
Calcedine dolomite, 25.9% MgO	120	—	—	—	—
Dolomite, 20.1% MgO	—	300	355	215	100
Sand	—	105	—	200	200
Total	2000	2000	2000	2000	2000

TABLE 2.—*Calcium carbonate equivalent of carbonate carbon*

COLLABORATOR	SAMPLE NUMBER									
	1		2		3		4		5	
	<i>per cent</i>	<i>lbs/ton</i>	<i>per cent</i>	<i>lbs/ton</i>	<i>per cent</i>	<i>lbs/ton</i>	<i>per cent</i>	<i>lbs/ton</i>	<i>per cent</i>	<i>lbs/ton</i>
1	9.60	192	14.70	294	18.48	370	11.06	221	5.50	110
	9.52	190	15.20	304	18.80	376	11.22	224	5.50	110
	—	—	15.10	302	18.30	366	—	—	—	—
Average	9.56	191	15.00	300	18.49	370	11.14	223	5.50	110
2	8.91	178	15.19	304	18.94	379	11.04	221	5.50	110
	8.91	178	—	—	—	—	—	—	—	—
Average	8.91	178	15.19	304	18.94	379	11.04	221	5.50	110
4	9.60	192	15.40	308	18.92	378	11.05	221	5.07	101
	9.69	194	15.31	306	18.74	375	10.98	220	5.59	112
Average	9.65	193	15.36	307	18.83	377	11.02	220	5.33	107

TABLE 3.—*Ash in acid-insoluble matter (acid-insoluble matter minus loss on ignition)*

COLLABORATOR	SAMPLE NUMBER									
	1		2		3		4		5	
	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton
1	1.59	32	7.95	159	4.27	85	13.76	275	14.16	283
	1.67	33	8.04	161	4.35	87	13.81	276	13.50	270
	—	—	—	—	—	—	—	—	13.89	278
Average	1.63	33	8.00	160	4.31	86	13.79	276	13.85	277
2	1.44	29	8.73	175	4.45	89	13.92	278	14.60	292
	1.66	33	8.61	172	3.47	89	13.61	272	14.37	287
Average	1.55	31	8.67	173	4.46	89	13.77	275	14.49	290
	1.67 ^a	33 ^a	8.88 ^a	178 ^a	4.56 ^a	91 ^a	13.74 ^a	275 ^a	14.58 ^a	292 ^a
3	1.68	34	9.07	181	4.49	90	13.67	273	14.11	282
4	1.31	26	8.71	174	4.54	91	13.88	278	14.10	282
	1.69	34	8.60	172	4.59	92	13.65	273	14.30	286
	1.70	34	8.56	171	4.46	89	—	—	—	—
Average	1.57	31	8.62	172	4.53	91	13.77	275	14.20	284

^a HCl (1+4) substituted for HCl (1+1).TABLE 4.—*Ash-free acid-insoluble organic matter*

COLLABORATOR	SAMPLE NUMBER									
	1		2		3		4		5	
	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton
1	1.78	36	1.38	28	4.68	94	2.14	43	2.78	56
	1.81	36	1.37	27	4.68	94	2.16	43	2.65	53
	—	—	—	—	—	—	—	—	2.76	55
Average	1.80	36	1.38	28	4.68	94	2.15	43	2.73	55
2	1.68	34	1.31	26	4.60	92	2.15	43	2.68	54
	1.77	35	1.33	27	4.63	93	2.21	44	2.86	57
Average	1.73	35	1.32	26	4.62	92	2.18	44	2.77	55
	2.18 ^a	44 ^a	1.44 ^a	29 ^a	5.60 ^a	112 ^a	2.67 ^a	53 ^a	3.04 ^a	61 ^a
3	1.82	36	1.32	26	4.71	94	2.27	45	2.79	56
4	2.06	42	1.22	24	4.44	89	2.19	44	2.77	55
	1.71	34	1.16	23	4.40	88	2.24	45	2.69	54
	1.67	33	1.20	24	4.64	93	—	—	—	—
Average	1.82	36	1.19	24	4.49	90	2.22	44	2.73	55

^a HCl (1+4) substituted for HCl (1+1).

TABLE 5.—*Ash-free water-insoluble organic matter*
(*Flotation separation*)

COLLABORATOR	SAMPLE NUMBER									
	1		2		3		4		5	
	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton	per cent	lbs/ton
1 ^a	3.66	73	.98	20	9.24	185	7.90	158	4.88	98
	3.67	73	1.07	21	9.65	193	7.77	155	4.26	85
Average	3.67	73	1.03	21	9.45	189	7.84	157	4.57	91
2	6.88	138	3.07	61	12.05	241	9.41	188	2.96	59
	3.01	60	.83	17	6.71	134	4.98	100	3.59	72
Average	4.95	99	1.95	39	9.38	188	7.20	144	3.28	66
	3.33 ^b	67 ^b	1.27 ^b	25 ^b	10.75 ^b	215 ^b	5.97 ^b	119 ^b	3.45 ^b	69 ^b
3	2.53	51	1.23	25	9.93	199	7.65	153	2.92	58
4	3.48	70	1.90	38	11.95	239	6.90	138	3.92	78
	3.26	65	2.00	40	11.95	239	7.00	140	3.86	77
Average	3.37	67	1.95	39	11.95	239	6.95	139	3.89	78

^a Except for sample Number 3, a 1-g rather than 5-g sample was used.^b 50-ml beaker used and overflowed into 400-ml beaker.

COMMENTS OF COLLABORATORS

Carbonate carbon or calcium carbonate equivalent

Florida Agricultural Department.—The CO₂ method is about perfect. I checked with control on four samples and a rerun on the other gave perfect check on my first run.

Bureau of Plant Industry, Soils, and Agricultural Engineering.—Reproducible results were readily obtained by the procedure as outlined.

Ash in acid-insoluble matter and ash-free acid-insoluble organic matter

Florida Agricultural Department.—The acid-insoluble ash and organic matter checked well. I made a third run using HCl (1+4) instead of HCl (1+1). The ash by this modification checked with the (1+1) but organic matter ran a little higher. The reason for using HCl (1+4) instead of (1+1) is to get away from breathing strong fumes of HCl. I believe the same thing can be accomplished by diluting the HCl (1+1) digestion when taken off the hot plate before filtering and washing. This is an empirical method. Just what is acid-insoluble organic matter? There is not a great deal of difference in results by HCl (1+1) and HCl (1+4). The latter is not objectionable to work with.

New Jersey Agricultural Experiment Station.—With careful and proper manipulation, concordant results can be obtained with the methods outlined.

Ash-free water-insoluble organic matter

California Department of Agriculture.—A 1-gram sample of four of the mixtures was used instead of a 5-gram sample as specified in the method, because a 5-gram sample of some of the materials gave suspensions which appeared too viscous to accomplish the desired result.

Florida Agricultural Department.—The water-insoluble method is satisfactory to

the point where the organic matter is skimmed off the calcium chloride flotation. At this point I had trouble skimming with suction. I tried overflowing it from a 50-ml. beaker placed inside a larger beaker. This worked better than skimming. The next trouble was filtering and washing. Three of the samples worked fairly well. The other two took 30 hours to complete. The final results do not check. I doubt the value of this determination even when the process is worked out.

New Jersey Agricultural Experiment Station.—Erratic results were obtained by the method as outlined due to the fact that not all the organic matter came to the surface in the 10 to 30 minutes specified in the procedure.

Bureau of Plant Industry, Soils, and Agricultural Engineering.—Non-ammoniated tobacco grades seemed to be the most difficult samples to handle by the flotation method, because more extraneous material (probably dolomite and superphosphate) is carried along with the organic material. The ignition step might be eliminated if the floated material is drenched with hot HCl (1 + 50) about twice while in the filter crucible. This acid treatment successfully gets rid of 80–90 percent of the extraneous material. The error then in the organic matter floated would not be excessive, and the weight of the sample prior to the ignition step probably would more nearly indicate the organic matter included in the formula than the ash-free water-insoluble matter, or loss on ignition.

DISCUSSION OF RESULTS

Carbonate carbon or calcium carbonate equivalent.—With the exception of the results for one sample the values reported by the collaborators were in good agreement, and no difficulty was reported in making the determination. Probably the method should be evaluated using individual materials, and mixtures formulated from materials of known carbonate content.

Ash in acid-insoluble matter and ash-free acid-insoluble organic matter.—The values reported by the various collaborators were in good agreement, but probably the ash obtained should receive an acid treatment to more nearly insure a more representative value of the silica content. The significance of the ash-free acid-insoluble organic matter has been questioned. This value is the ignition loss obtained on ashing the total acid-insoluble material, and presumably represents difficulty decomposable organic matter of little or no immediate fertilizer value. Additional study should be given to a method for determining acid-insoluble ash.

Ash-free water-insoluble organic matter.—The erratic results obtained and considerable difficulty experienced by the collaborators in making this determination indicate the need for an improved procedure for direct separation of organic material from mixtures. Possibly reducing the sample size, washing the floated material with dilute acid, and omitting the ignition step would accomplish the objective. A comparison, however, should be made between such a revised procedure and the possibility that the ignition loss of the water-insoluble portion of the original sample would provide a satisfactory measure of the organic matter.

RECOMMENDATION

It is recommended that the work be continued.

CONTRIBUTED PAPERS

A STUDY OF CLARIFICATION METHODS IN THE DETERMINATION OF SUGARS IN WHITE POTATOES

USE OF ION-EXCHANGE RESINS

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In the preparation of potato chips (6, 16, 17, 20) and dehydrated white potatoes (5, 12), the reducing-sugar content of the potato is a major factor in the formation of objectionable color. Research on potato processing at this laboratory has therefore made it necessary to determine sugars on a large number of samples. A rapid, accurate method of analysis for sugars would facilitate this work.

The authors have found that each type of plant material must be investigated to determine the most satisfactory procedure for estimation of sugar content. This conclusion is in agreement with that of the Referee, Elroy J. Miller, and co-workers (18).

By comparison of sugar values obtained by different methods of analysis, it was concluded that non-sugar reducing substances introduced significant errors in some of the methods. Therefore, the emphasis of the investigation was directed towards the removal of these substances, hereinafter referred to as clarification.

The substitution of ion-exchange resins for neutral lead acetate and carbon treatments, for the removal of non-sugar reducing materials from potato extracts, resulted in a major improvement in the clarification procedure.

Three methods were used to determine sugars in clarified extracts from raw potatoes, and four methods were used for extracts from dehydrated potatoes (ca. 6-8 per cent moisture). The methods used and the results obtained are presented in this report.

PROCEDURES

(1) *Extraction of sugars:* The raw potatoes were quickly peeled, sliced, chopped finely, and added to sufficient boiling 95 per cent ethyl alcohol (freshly redistilled), to give with the water in the potatoes an approximate alcohol concentration of 80 per cent. Calcium carbonate (1-2 g) was added and the mixture was heated 1 hour, with frequent stirring, on a steam bath. The supernatant liquor was decanted through a folded filter paper. The

¹ One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

residue was covered with 80 per cent alcohol and reheated for 0.5 hour and the liquid was filtered as before. The residue was transferred to a Waring Blendor cup² and sufficient 80 per cent alcohol was added to make a slurry. After blending for about 5 minutes, the mixture was transferred to a Soxhlet extractor and the extraction process was allowed to continue for 0.5 hour. The extract was removed, replaced with fresh 80 per cent alcohol, and the extraction continued for 4-5 hours. All of the extracts were combined and stored in the dark until used.

The dehydrated potatoes were ground to pass a 40-mesh screen. The extraction process was similar to that used for fresh potatoes, with the exception that the blending step was omitted and 80 per cent alcohol was used throughout the procedure.

The complete extraction of the sugars was not demonstrated, but for the purpose of comparison of methods of analysis on each extract, this was not necessary.

(2) *Clarification of the potato extracts:* The extract was evaporated on a steam bath to remove the alcohol (1), and the residual water solution was diluted to a definite volume. Three methods were used to clarify aliquots of this water solution: (a) An excess of saturated neutral lead acetate solution was added to the water solution, the lead precipitate was removed by filtration, and the excess lead was removed with disodium phosphate solution. (b) An aliquot of the solution obtained in (a) was treated at room temperature with carbon to remove all color (5 mg. of carbon per ml. of solution for 10 minutes). (c) The water solution was passed through a set of ion-exchange resin columns. For brevity, these three methods of clarification will be referred to as the lead, lead-carbon, and resin treatments.

The ion-exchange columns used in this study have been illustrated and described elsewhere (19). One tube contained 15 g. of the cation-exchange resin, Amberlite IR-100 H-AG,² and the other tube contained 20 g. of the acid-binding resin, Amberlite IR-4 B-AG. The columns were back-washed with distilled water for 1-2 hours just before they were used. Two 30-ml. aliquots of the water solution were passed slowly through the IR-100 H-AG, and then through the IR-4 B-AG and discarded, to eliminate the dilution error. Then, 100-125 ml. of the water solution to be used for sugar analysis were passed through the columns at the rate of approximately 3 ml. per minute. The amount of solution passed through the columns represented about 70 g. of raw, or 30 g. of dehydrated, potatoes. Although the capacity of the columns was not determined, no breakthrough was observed. Pure solutions of known concentrations of dextrose, levulose, and sucrose were passed through the resin columns in the same manner as the potato solutions with no change in sugar concentration.

² The mention of manufacturers and commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others not mentioned.

Since the insoluble material in the water solution from dehydrated potatoes interfered with the effectiveness of the resins, the procedure was modified by adding 2 g. of Celite Analytical Filter-Aid to the water solution and filtering with suction through a Büchner funnel containing a filter paper covered with a thin mat of Celite. Experiments with pure solutions of dextrose, levulose, and sucrose showed that the filter aid did not adsorb any perceptible amounts of these sugars. This is in agreement with Waldron *et al.* (18).

(3) *Inversion*: The clarified solutions were adjusted to a pH of 4.8–5.1 with 10 per cent acetic acid solution, and total sugars were determined after treatment of the solutions with invertase (Difco Laboratories, Detroit, Mich.) overnight at room temperature. The extracts were hydrolyzed prior to carbon treatment, to prevent sucrose adsorption (4). It was necessary to add benzoic acid to the untreated and resin-treated solutions before hydrolysis, to prevent microbiological growth which lowered the sugar values.

(4) *Analysis of the clarified extracts*: Sugars were determined on the clarified solutions by the following methods: (a) A procedure that will be referred to as the macro method (2), whereby 50–150 mg. of reducing sugars are reacted with cupric salts, the cuprous oxide is filtered off and dissolved in nitric acid, and the amount of copper is determined by thio-sulfate titration. This method requires about 50 times the amount of sugar necessary for the micro methods, and it was included primarily as a reference method (9). (b) A procedure that will be referred to as the A.O.A.C. Somogyi method (3, 14), whereby 1–2 mg. of reducing sugars are reacted with cupric salts in the presence of potassium iodide and potassium iodate, and the iodine liberated on acidification is determined by thiosulfate titration. (c) A procedure that will be referred to as the Somogyi modified method (15), which is procedure (b) as improved by Somogyi. (d) A procedure that will be referred to as the Hassid method (7, 8), whereby 1–3 mg. of reducing sugars are reacted with alkaline ferricyanide, and the ferrocyanide formed is titrated in acid solution with ceric sulfate, using the indicator Setopalin C. The sugar values given in the tables are the averages of closely agreeing duplicates.

DISCUSSION OF RESULTS

A previous investigation with different commercially available decolorizing carbons showed that their sugar adsorptive properties varied (4). Four of these carbons, which did not absorb measurable quantities of levulose or dextrose, were selected for use in the clarification procedure on two raw potato samples, one of high and one of low reducing-sugar content. The data are given in Table 1. Any apparent differences in reducing-sugar content resulting from the carbon treatment were due to the removal of non-sugar reducing materials. All of the carbon-treated solu-

tions were water clear, but the sugar values obtained by the Hassid method were higher than those obtained by the macro method, and these differences were greater, proportionately, in the potato of low reducing-sugar content. Throughout this investigation, it has been observed that the removal of color does not indicate the complete removal of non-sugar reducing substances. With raw potatoes, the macro method gave essentially the same sugar values, with or without lead-carbon treatment, irrespective of the type of carbon used. Since the lead-carbon treatment was

TABLE 1.—*Effect of decolorizing carbons on the determination of reducing sugars in raw white potatoes*¹

SAMPLE NO.	METHOD OF ANALYSIS	PER CENT REDUCING SUGARS ²				
		NO CARBON TREATMENT	DECOLORIZING CARBON			
			G60 ³	KB ⁴	B&A ⁴	325 ⁵
A	Macro (2)	0.195	0.192	0.186	0.197	0.199
	Hassid (7, 8)	0.274	0.224	0.232	0.242	0.256
B	Macro (2)	1.07	1.05	1.05	1.05	1.05
	Hassid (7, 8)	1.10	1.10	1.08	1.11	1.11

¹ Prior to the carbon treatment, the potato extracts (after the removal of alcohol) were treated with excess neutral lead acetate solution and the excess lead was removed with disodium phosphate, as described under Procedures, clarification step (a).

² Calculated as dextrose.

³ Darco Corp., New York, N. Y.

⁴ Baker & Adamson Code No. 1551, General Chemical Co., New York, N. Y.

⁵ Animal Charcoal 325 Mesh, Consolidated Chem. Ind. Inc., San Francisco, Calif.

not satisfactory with the Hassid method, it was necessary to find a different method of clarification.

Partridge and Westall (10) used ion-exchange resins for the removal of "bases and cations" and "acids" from extracts of biological materials in their procedure for the identification of sugars by chromatography. Putnam *et al.* (11) found ion-exchange resins useful for removing minerals and organic and amino acids from solutions in the preparation of radioactive carbon-labeled sugars. Serbia (13) found that a mixture of ion-exchange resins added to cane molasses would remove the non-sugar reducing materials that interfered with the Lane-Eynon method of analysis for sugars. The authors tested ion-exchange resins, in glass columns, for the removal of non-sugar reducing materials from white potato extracts. This method of clarification was compared with the lead and with the lead-carbon procedures (see (2)) on extracts prepared from raw and dehydrated potatoes (Tables 2 and 3).

When solutions prepared from raw potatoes were clarified by resin treatment, all of the methods gave, within experimental error, the same sugar values. These values were in marked contrast to those obtained by

TABLE 2.—Percentages of reducing and total sugars in extracts of raw white potatoes¹ as determined by different methods of clarification and evaluation

SAMPLE NO.	METHOD OF CLARIFICATION ²	METHODS USED TO EVALUATE SUGARS					
		REDUCING SUGARS ³			TOTAL SUGARS ³		
		A.O.A.C.		HABIB (7, 8)	A.O.A.C.		HABIB (7, 8)
		MACRO (2)	SOMOGYI (3, 14)		MACRO (2)	SOMOGYI (3, 14)	
1	Resin	0.48	0.50	0.47	1.39	1.41	1.41
	Lead	0.49	0.51	0.55	1.47	1.49	1.53
	Lead-carbon	0.50	0.52	0.53	1.45	1.51	1.51
2	Resin	0.29	0.31	0.32	0.69	0.73	0.71
	Lead	0.31	0.35	0.39	0.70	0.78	0.79
	Lead-carbon	0.32	0.36	0.36	0.70	0.74	0.77
3	Resin	0.26	0.27	0.28	0.92	0.91	0.92
	Lead	0.28	0.29	0.35	0.93	0.95	0.98
	Lead-carbon	0.27	0.29	0.32	0.93	0.95	0.97
4	Resin	0.61	0.59	0.62	1.30	1.31	1.31
	Lead	0.61	0.67	0.67	1.30	1.37	1.36
	Lead-carbon	0.61	0.65	0.64	1.29	1.35	1.33
5*	Resin	0.04	0.05	0.07	0.25	0.24	0.26
	Lead	0.05	0.07	0.14	0.26	0.29	0.33
	Lead-carbon	0.04	0.07	0.11	0.26	0.28	0.31
6	Resin	0.38	0.37	0.40	0.82	0.82	0.84
	Lead	0.40	0.43	0.50	0.85	0.88	0.94
	Lead-carbon	0.42	0.45	0.48	0.85	0.89	0.93
	No treatment	0.39	0.40	0.50			
7*	Resin	0.12	0.12	0.13	0.37	0.36	0.37
	Lead	0.15	0.16	0.20	0.37	0.37	0.45
	Lead-carbon	0.15	0.16	0.19	0.38	0.41	0.42
	No treatment	0.11	—	0.21			
8	Resin	0.26	0.28	0.26	0.48	0.49	0.46
	Lead	0.28	0.30	0.35	0.48	0.55	0.56
	Lead-carbon	0.27	0.31	0.32	0.49	0.53	0.54
	No treatment	0.27	0.32	0.34			
9	Resin				0.64	0.66	0.63
	Lead				0.62	0.70	0.72
	Lead-carbon				0.63	0.69	0.69
	No treatment				0.64	0.69	0.73
10	Resin				0.32	0.34	0.34
	Lead				0.33	0.39	0.42
	Lead-carbon				0.34	0.39	0.38
	No treatment				0.33	0.40	0.44
11	Resin				0.41	0.42	0.42
	Lead				0.42	0.48	0.51
	Lead-carbon				0.43	0.47	0.47
	No treatment				0.41	0.49	0.53

¹ The raw potatoes were purchased at various times over a period of six months at local vegetable markets. It was impossible to ascertain the potato variety or the storage history of each purchase.

² Resin = water-solution of potato extract passed through the ion-exchange columns.

Lead = water-solution of potato extract treated with excess neutral lead acetate solution and the excess lead removed with disodium phosphate.

Lead-carbon = same as "Lead" treatment, plus additional 10-minute treatment with Baker & Adamson Code No. 1551 decolorising carbon (5 mg. carbon per ml. of solution).

No treatment = No chemical treatment of the water-solution of the potato extract.

³ Calculated as dextrose.

* The reducing-sugar content of Samples No. 5 and No. 7 was below the minimum amount specified for the A.O.A.C. macro method.

TABLE 3.—Percentages of total sugars in extracts of dehydrated white potatoes as determined by different methods of clarification and evaluation

SAMPLE NO.	DESCRIPTION OF SAMPLE	METHOD OF CLARIFICATION ¹	METHODS USED TO EVALUATE SUGARS ²			
			A.O.A.C.		SOMOGYI MODIFIED (15)	HASSID (7),(8)
			MACRO (2)	SOMOGYI (3),(14)		
12	Maine Green Mountain stored 3 yr. @ 34°F. in nitrogen	Resin	1.57	1.49	—	1.60
		Lead	1.62	1.68	—	1.89
		Lead-carbon	1.62	1.66	—	1.78
		No treatment	1.61	1.63	—	1.85
13	Maine Katahdin stored 3 yr. @ 34°F. in nitrogen	Resin	0.51	0.52	—	0.52
		Lead	0.54	0.62	—	0.82
		Lead-carbon	0.55	0.60	—	0.77
		No treatment	0.50	0.56	—	0.79
14	California Russet stored 4 mo. @ 75°F. in air	Resin	1.00	1.06	—	1.03
		Lead	1.09	1.12	—	1.40
		Lead-carbon	1.09	1.10	—	1.31
		No treatment	0.99	1.21	—	1.34
15	Idaho Russet stored 4 yr. @ 34°F. in air	Resin	1.59	1.53	1.63	1.54
		Lead	1.72	1.83	1.84	1.99
		Lead-carbon	1.72	1.73	1.84	1.88
		No treatment	1.72	1.66	1.83	2.04
16	Idaho Russet treated with 200 p.p.m. SO ₂ before dehydration. Stored 3 yr. @ 34°F. in nitrogen	Resin	0.97	0.93	0.94	0.99
		Lead	1.01	1.08	1.07	1.16
		Lead-carbon	1.02	1.06	1.07	1.10
		No treatment	1.02	1.07	1.09	1.20
17	Oregon Netted Gem treated with 400 p.p.m. SO ₂ before dehydration. Stored 4 yr. @ 75°F. in nitrogen	Resin	1.10	1.02	1.11	1.09
		Lead	1.27	1.40	1.39	1.58
		Lead-carbon	1.29	1.38	1.35	1.50
		No treatment	1.31	1.46	1.41	1.62
18	Oregon Netted Gem stored 4 yr. @ 75°F. in nitrogen	Resin	0.93	0.91	0.93	0.92
		Lead	1.14	1.19	1.19	1.43
		Lead-carbon	1.14	1.18	1.18	1.33
		No treatment	1.06	1.22	1.19	1.42
19	Variety unknown, commercially dehydrated. Stored 4 yr. @ 34°F. in air	Resin	2.81	2.67	2.85	2.73
		Lead	3.12	3.23	3.26	3.33
		Lead-carbon	3.15	3.27	3.23	3.25
		No treatment	3.13	3.12	3.25	3.29

¹ See Footnote No. 2, Table 2.² Calculated as dextrose.

the different methods when lead or lead-carbon clarification was used. For example, in sample No. 8, when lead clarification was used, the reducing-sugar value obtained by the Hassid method was 30 per cent higher than by the macro method; when carbon treatment was included, the

value was 18 per cent higher. It is well demonstrated throughout Table 2 that, under the conditions of procedure, lead or lead-carbon clarification did not adequately remove the non-sugar reducing materials that affected the sugar values obtained by the Hassid method. The fact that the macro method gave the same values, regardless of the method of clarification, suggested the analysis of the untreated potato extract. The results showed (Table 2) that no clarification was necessary in the analysis for sugars in the raw potato by this method. The specificity of the A.O.A.C. macro method recommends it when sufficient sugar is available for the analysis.

When the comparison of procedures was made on dehydrated potatoes, only total sugars were determined, because more material was required than was suitable for handling to provide sufficient reducing sugars for all the methods. It should be noted, however, that the non-sugar reducing materials would be a much larger portion of the values obtained for reducing sugars than of those for total sugars (see example given later in this report).

All of the methods gave comparable sugar values for dehydrated potatoes when resin treatment was used (Table 3). The micro methods gave much higher sugar values for the lead and lead-carbon-treated samples than for the resin-treated samples. For example, in sample No. 17 (Table 3), the Somogyi modified method gave values 25 per cent and 21 per cent higher for the lead and lead-carbon treatments, respectively, and the Hassid method gave values 45 per cent and 37 per cent higher. Additional analyses were made on this sample with other decolorizing carbons (Darco KB and Darco G60) in the lead-carbon clarification procedure. Nevertheless, sugar values were consistently high in comparison with resin treatment.³ The magnitude of this error is much greater in the determination of the reducing sugars, since the same non-sugar reducing materials are included as in the determination of total sugars. For example, the reducing-sugar values obtained for sample No. 17 by the Hassid method were 0.42 per cent when resin treatment was used, and 0.93 per cent with lead-carbon treatment. In this same experiment, the A.O.A.C. Somogyi method gave 0.42 per cent, and the Somogyi modified method gave 0.38 per cent, reducing sugars with resin treatment, against 0.55 per cent and 0.65 per cent, respectively, with lead-carbon treatment.

The data obtained on the dehydrated potato by the macro method were of considerable interest, since additional reducing materials appeared to have been formed during the dehydration of the potato. This was suggested by the fact that the sugar values were, in general, lower with resin treatment than with the other methods of clarification. For raw potatoes, the sugar values determined by the macro method were the same, with or without clarification.

³ If the quantity of carbon is materially increased, adsorption of dextrose and levulose can be expected (4).

CONCLUSIONS

No clarification step was necessary when the A.O.A.C. macro method was used for the analysis of sugars in raw potatoes.

The removal of color was not necessarily an indication of the complete removal of non-sugar reducing materials.

Non-sugar reducing materials in raw potatoes which interfered with the micro methods of analysis, and in dehydrated potatoes which interfered with both macro and micro methods, were not completely removed by neutral lead acetate or decolorizing carbon.

When ion-exchange resins were used to remove non-sugar reducing materials, concordant results were obtained by all of the methods studied. Furthermore, the sugar values were lower than those obtained with other methods of clarification. (Experiments with pure solutions of dextrose, levulose, and sucrose, in concentrations comparable to those used in this investigation, showed that the resins did not adsorb these sugars.)

The greater accuracy of the sugar values obtained by the use of ion-exchange resins in this study warrants the investigation of their use in sugar determinations on other biological materials. Such studies are in progress at this laboratory.

ACKNOWLEDGMENT

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STABILIZATION OF STANDARD CAROTENE SOLUTIONS

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In the final measurement of the color of a carotene solution, in the determination of provitamin A pigments, it is necessary to use some apparatus such as an optical or a photoelectric colorimeter or a spectrophotometer. Very frequently it is necessary to recalibrate or check the electrical instruments and, in the case of optical colorimeters, a standard solution is required for comparison with the unknown solution.

A Coleman Model 11 Universal spectrophotometer is used for colorimetry in the author's laboratory. In order to establish a standard curve, crystalline carotene obtained from General Biochemicals, Inc., Chagrin Falls, Ohio, is further purified by precipitation with methanol (1). This purified carotene is used to make up standard solutions of varying concentrations. Transmittance readings from these solutions are then plotted on graph paper as a colorimetric curve. Petroleum ether or Skellysolve B is used as a solvent.

Pure carotene solutions deteriorate rapidly especially when exposed to daylight at room temperature. In fact, at the end of three days a standard carotene solution initially containing 100 micrograms per 100 ml. stored under the above conditions showed an 8 per cent loss of its potency; at the end of one week's storage, a 14 per cent loss; and after 12 weeks, bleaching and oxidation of the carotene had progressed to such an extent that there was only a negligible quantity of the original pigment left in the solution.

Therefore, because of the labile character of carotene, standard solutions cannot be stored for future use, but must be made up fresh as needed. Furthermore, crystalline carotene deteriorates quite rapidly after the sealed ampules, in which it is contained, are broken open.

Obviously a means of stabilizing standard carotene solutions would be desirable from the standpoint of both time and cost economy. Although a dilute solution of potassium dichromate has been used fairly successfully as a standard comparison solution in optical colorimeters, its use as a standard in photoelectric colorimeters is not recommended. The maximum light absorption for a potassium dichromate solution is at a much lower wave length (390 millimicrons) than for a carotene solution. Therefore,

the most ideal and practical method would be to employ a stabilized carotene solution for establishment of a colorimetric curve, as well as for a reference and comparison standard.

Several reports have been made on the antioxidant effect of tocopherols. Buxton (2) used mixed tocopherols as antioxidants for Vitamin A in fish liver oils. Hickman, *et al.*, (4) showed that Vitamin E (tocopherols) helped prevent the destruction of Vitamin A and carotene in the digestive tract. Quackenbush, *et al.*, (5) stated that tocopherols retard the oxidation of carotene.

In the author's laboratory it has been found that mixed tocopherols (alpha, beta, and gamma) are good antioxidants, if used in proportionally larger quantities, for stabilization of Vitamin A and carotene in solutions.

TABLE 1.—*Solutions stored in daylight (not direct sunlight) at room temperature (20°C.)*

MICROGRAMS OF CAROTENE PER 100 ML.	PER CENT REMAINING								AFTER CHROMATO- GRAPHING
	START	3 DAYS	1 WEEK	3 WEEKS	5 WEEKS	7 WEEKS	9 WEEKS	12 WEEKS	
100 no Tocoph.	100	92	86	64	40	28	16	10	Negligible
200 no Tocoph.	100	95	90	77	70	66	50	42	30
100 + .1 mg. Tocoph.	100	100	96	95	90	80	68	60	55
200 + .1 mg. Tocoph.	100	100	98	96	95	90	85	79	76
100 + .5 mg. Tocoph.	100	100	100	96	94	90	88	80	75
200 + .5 mg. Tocoph.	100	100	100	100	97	94	90	88	87
100 + 1 mg. Tocoph.	100	100	100	100	100	100	100	100	97
200 + 1 mg. Tocoph.	100	100	100	100	100	100	100	100	98
100 + 5 mg. Tocoph.	100	100	100	100	100	100	100	100	100
200 + 5 mg. Tocoph.	100	100	100	100	100	100	100	100	95

Hence it was deemed necessary to investigate the possibility of tocopherols protecting carotene solutions, under various conditions, for at least 12 weeks. Because of the fact that carotene is sensitive to oxidation, and light, identical sets of solutions were stored in daylight (not direct sunlight) at room temperature (20°C.), in the dark at room temperature, and in the refrigerator.

In the preparation of the solutions, the tocopherols used were derived from a concentrate of mixed tocopherols produced by Distillation Products, Inc., which is claimed to contain 220 mg. of mixed tocopherols per gram. Our assay by the method of Devlin and Mattill (3), which is a modification of the older Emmerie and Engel method, showed 230 mg. mixed tocopherols per gram.

The solutions were stored for 12 weeks, and at regular intervals the color of each was measured in the spectrophotometer at 440 millimicron wave length. At the end of the 12-week period the solutions were chro-

TABLE 2.—Solutions stored in dark at room temperature (20°C.)

[illegible]

matographed by the Wall-Kelley chromatographic procedure (6) to remove oxidation, and other degradation, products.

In Tables 1, 2, and 3 are given results of storage of 100 ml. solutions containing either 100 or 200 micrograms of carotene each, plus varying amounts of tocopherols.

SUMMARY AND CONCLUSIONS

By incorporating small quantities of mixed tocopherols or pure alpha tocopherol in petroleum ether solutions of purified carotene, an antioxidant effect is attained by which the carotene is preserved from deterioration for as long as 12 weeks. Such carotene solutions therefore, can be used as colorimetric standards over such a period. Without the use of tocoph-

TABLE 3.—Solutions stored in refrigerator

[illegible]

erols in these solutions, destruction of the carotene is so rapid that they are no longer reliable after two or three days.

In order to stabilize standard carotene solutions it is necessary to add from 10 to 50 times as much tocopherols as there is carotene present. The use of more than 5 mg. of tocopherols per 100 ml. of petroleum ether may produce a measurable color which would be spuriously read as carotene.

The destruction of carotene in higher potency solutions is not as rapid as in dilute solutions, possibly due to an antioxidant effect by the carotene itself.

The conditions of storage of the stabilized carotene reference standards are not too consequential, although it is no doubt desirable to protect them from light as much as possible, and from heat.

Recovery of carotene from a solution which has been passed through a chromatographic column is not always 100 per cent, due to losses by manipulation, incomplete elution, oxidation, etc. However, in solutions of carotene which have been stored for long periods of time, and destruction of the provitamin A pigment is evident, the magnesia adsorption column removes degradation products.

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REFRACTIVE INDICES OF LACTOSE SOLUTIONS

By F. W. ZERBAN and JAMES MARTIN (New York Sugar Trade Laboratory, New York, N. Y.)

Emma J. McDonald (1) has recently published five-place tables of the refractive indices of lactose hydrate solutions at 20° and 25°C., for concentrations up to 16 per cent, based on weights in vacuo; also four-place tables for concentrations from 16 to 36 per cent at 25°; and for the entire concentration range at 15°. Similar measurements to the fifth decimal place were made in this laboratory during the spring of 1947, at 20°, upon solutions containing up to 32 per cent of lactose hydrate, based on weights in air. A Bausch and Lomb Precision Refractometer was used in this work and the same procedure was followed as previously described for measurements upon dextrose and invert sugar solutions (2). The lactose was prepared by recrystallizing the commercial product, by

the method given in National Bureau of Standards Circular C-440, p. 467, until the specific conductance of a 5-per cent solution did not decrease further. This required six recrystallizations. The purified sugar was first dried in air, and then in a thin layer at 50°C. in an air oven. The residual moisture in the hydrate was determined by heating for two hours at 100°C. at atmospheric pressure. It was found to equal 0.038 per cent, and this was corrected for in weighing out the sugar for each experiment. All solutions were allowed to stand for 24 hours to complete mutarotation. The results of the measurements upon 30 solutions are given in the second column of Table 1.

TABLE 1.—*Refractive indices of lactose hydrate solutions; observed data and values calculated from Equation 1*

CONCN. OF LACTOSE HYDRATE, WEIGHTS IN AIR, PER CENT	n_D^{20} OBSERVED	n_D^{20} CALCD. BY EQUATION 1	DEVIATION FROM FORMULA	SQUARE OF RESIDUALS $\times 10^{-10}$
1.004	1.33437	1.33439	-0.00002	4
1.973	1.33580	1.33577	+0.00003	9
3.002	1.33721	1.33723	-0.00002	4
4.024	1.33869	1.33869	0.00000	0
5.095	1.34020	1.34025	-0.00005	25
6.045	1.34166	1.34164	+0.00002	4
7.107	1.34323	1.34321	+0.00002	4
7.558	1.34389	1.34388	+0.00001	1
9.286	1.34646	1.34647	-0.00001	1
10.095	1.34771	1.34769	+0.00002	4
10.962	1.34901	1.34901	0.00000	0
11.666	1.35007	1.35003	-0.00004	1
13.349	1.35267	1.35268	-0.00001	1
14.370	1.35427	1.35426	+0.00001	1
15.192	1.35554	1.35555	-0.00001	1
16.288	1.35726	1.35728	-0.00002	4
17.360	1.35897	1.35895	+0.00002	4
17.620	1.35938	1.35939	-0.00001	1
17.709	1.35953	1.35953	0.00000	0
18.426	1.36071	1.36068	+0.00003	9
20.048	1.36332	1.36329	+0.00003	9
20.483	1.36394	1.36400	-0.00006	36
20.856	1.36464	1.36461	+0.00003	9
22.602	1.36747	1.36747	0.00000	0
23.675	1.36925	1.36925	0.00000	0
25.593	1.37255	1.37247	+0.00008	64
25.818	1.37275	1.37285	-0.00010	100
27.795	1.37624	1.37624	0.00000	0
30.207	1.38046	1.38044	+0.00002	4
32.076	1.38380	1.38378	+0.00002	4
				304

From these data the following equation was computed by the method of averages:

$$n_D^{20}, \text{ lactose hydrate} = 1.33299 + 0.0013887 p + 0.0000076668 p^2 - 0.00000012179 p^3 + 0.0000000022419 p^4 \quad (1)$$

The deviations from this formula are very small below the saturation point, and exceed 0.00005 only at a few concentrations in the super-saturation range above 20 per cent, where it is difficult to prevent incipient crystallization. For the same reason attempts to prepare solutions above about 32 per cent concentration were unsuccessful.

The sum of the squares of the residuals for the 30 experiments equals 304×10^{-10} , which is not as good as the figure 190×10^{-10} , found for our dextrose equation, based on 23 experiments; but much better than the figure 1550×10^{-10} for Saunders's levulose equation, based on the data of Jackson and Mathews, comprising 32 experiments (1). The respective variances for these three series of experiments are thus: 10.48×10^{-10} , 8.64×10^{-10} and 50.0×10^{-10} , respectively. The respective standard errors of estimate are then: 0.000032, 0.000029, and 0.000071.

Refractive indices of lactose hydrate solutions at 20° had previously been reported also by Tolman and Smith (3), and by Rothenfusser and Kotschopoulos (4). Table 2 shows a comparison of the various data, all based on weights in air. McDonald's concentration figures have been converted to this basis by calculation, and her refractive index values for concentrations above 16 per cent have been interpolated between those for 25° and 15°.

It is noted that the authors' figures are a few units of the fifth decimal place lower than McDonald's up to a concentration of 12 per cent lactose hydrate, and one or two units higher between 14 and 16 per cent. They check exactly to the fourth decimal place for concentrations from 17 to 32 per cent. On the whole, the agreement between the two series of results is very satisfactory, considering that the measurements were made by different observers using different instruments, and that McDonald computed quadratic equations by the method of least squares, while the authors used a quartic equation derived by the method of averages. The results of Rothenfusser and Kotschopoulos are slightly lower than those of the authors up to a concentration of 4 per cent, and above that become increasingly lower. The data of Tolman and Smith check, within about one unit of the fourth decimal place, with those of McDonald and of the authors.

Previous literature also gives figures for the refractive indices of lactose solutions, measured at 17.5°C. by Stolle (5), at 18.5°C. by Golse (6), and at 25°C. by Pulvermacher (7). These are compared in Table 3 with the values computed from McDonald's results. To find $n_D^{17.5}$ based on her values, the average between her figures for n_D^{20} and n_D^{16} was taken. The $n_D^{18.5}$ had to be computed by linear interpolation between n_D^{16} and n_D^{25} ,

TABLE 2.—*Comparison of various data for the refractive indices of lactose hydrate solutions at 20°C.*

CONCN. OF LACTOSE HYDRATE, WEIGHTS IN AIR, PER CENT	MCDONALD	MARTIN EQUATION 1	ROTHENFUSSE AND KOTSCHOPOULOS	TOLMAN AND SMITH
0.0	1.33299	1.33299	1.33300	1.3330
0.5	1.33370	1.33369	1.33368	
1.0	1.33440	1.33439	1.33439	1.3343
1.5	1.33511	1.33510	1.33507	
2.0	1.33583	1.33580	1.33578	1.3357
2.5	1.33654	1.33652	1.33649	
3.0	1.33726	1.33722	1.33720	
3.5	1.33798	1.33795	1.33791	
4.0	1.33871	1.33866	1.33862	
4.5	1.33943	1.33939	1.33930	
5.0	1.34016	1.34011	1.33999	
5.13	1.34035	1.34030		1.3402
5.5	1.34089	1.34084	1.34071	
6.0	1.34162	1.34158	1.34145	
7.0	1.34310	1.34305		
8.0	1.34458	1.34454		
9.0	1.34606	1.34604		
10.0	1.34757	1.34754		
10.13	1.34776	1.34774		1.3477
11.0	1.34908	1.34907		
12.0	1.35061	1.35059		
13.0	1.35214	1.35214		
14.0	1.35368	1.35369		
15.0	1.35523	1.35525		
15.13	1.35544	1.35546		1.3555
16.0	1.35680	1.35682		
17.0	1.3584	1.35840		
18.0	1.3600	1.36000		
19.0	1.3616	1.36160		
20.0	1.3632	1.36322		
21.0	1.36485	1.36484		
22.0	1.3665	1.36648		
23.0	1.36815	1.36813		
24.0	1.3698	1.36980		
25.0	1.3715	1.37147		
26.0	1.3732	1.37316		
27.0	1.3749	1.37487		
28.0	1.3766	1.37659		
29.0	1.37835	1.37833		
30.0	1.3801	1.38008		
31.0	1.38185	1.38185		
32.0	1.3836	1.38364		
34.0	1.3872			
36.0	1.3908			

because the values for 20° were not determined by her for concentrations above 16 per cent lactose. Pulvermacher's original results are for anhydrous lactose, and his concentrations were first converted into corresponding concentrations of lactose hydrate. McDonald's values for n_D^{25} were calculated by her equation 5 for concentrations up to 16 per cent lactose, and by her equation 6 for concentrations above that figure.

TABLE 3.—*Comparison of refractive indices of lactose hydrate solutions at 17.5, 18.5, and 25°*

CONCN. OF LACTOSE HYDRATE			CONCN. OF LACTOSE HYDRATE			CONCN. OF LACTOSE HYDRATE		
$n_D^{17.5}$			$n_D^{18.5}$			n_D^{25}		
STOLLE		MCD.	GOLSE		MCD.	PULV.		MCD.
per cent			per cent			per cent		
1.0007	1.33473	1.3346	4.65	1.3400	1.3397	1.35	1.3350	1.33441
1.9950	1.33588	1.3360	7.56	1.3433	1.3440	2.93	1.3380	1.33667
3.9489	1.33873	1.3388	14.35	1.3551	1.3544	6.10	1.3423	1.34127
7.7931	1.34448	1.3445	18.47	1.3604	1.3609	12.27	1.3517	1.35047
			22.51	1.3670	1.3675	17.96	1.3605	1.3594
			26.97	1.3746	1.3750	24.61	1.3716	1.3703

Table 3 shows satisfactory agreement between Stolle's and McDonald's values, but those of Golse are from 0.0007 lower to 0.0007 higher than McDonald's, and are evidently unreliable. Pulvermacher's figures are from 0.0006 to 0.0013 higher throughout, the discrepancies averaging 0.0011. But Pulvermacher's results for n_D^{25} of sucrose also average 0.0009 higher than those internationally accepted, and there must have been a systematic error in his measurements.

SUMMARY

The refractive indices of lactose hydrate solutions, containing up to 32 per cent of this sugar, have been determined at 20°C. to the fifth decimal place with a Bausch and Lomb Precision Refractometer. The results agree satisfactorily with those reported by McDonald, given by her to five decimal places in the range up to 16 per cent concentration, and interpolated from her figures for n_D^{25} and n_D^{15} at concentrations between 16 and 36 per cent. Results obtained by previous authors are compared with the more recent ones and critically discussed.

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DETERMINATION OF GLUCOSE, GALACTOSE, AND RHAMNOSE IN MIXTURES*

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In a study of the flavonol glycosides of buckwheat and other plants, it was desirable to determine the carbohydrates in their hydrolysates, both qualitatively and quantitatively. Chemical methods for the separation of glucose, rhamnose, and galactose (6) were not suitable because they were tedious, quantitatively inaccurate, and not successful in identifying sugars present in trace amounts. Polarimetric methods (3) for analyses of the possible combinations were not applicable because of interfering substance resulting from the hydrolysis, and also because of the limited quantities of most of the materials to be analyzed. A combination of methods—namely, filter paper chromatography (5), copper reduction (4), and yeast fermentation (1, 2, 7, 8)—was finally employed, with complete success. Although the procedure to be presented deals exclusively with mixtures of glucose, rhamnose, and galactose, the principles may be applied in analyses of mixtures of other sugars.

METHODS

Hydrolysis.—Several methods of hydrolysis were employed. However, boiling under reflux for 2 hours in 2.5 per cent sulfuric acid, or for 1 hour in 5 per cent sulfuric acid, gave the best recoveries of quercetin and sugars from rutin. Before analysis, the sulfuric acid was neutralized with sodium hydroxide.

Copper Reduction.—The sugar analyses were made by Schoorl's method (4).

Paper Chromatography.—The method of Partridge (5) was used. The solvent employed was the mixture of 40 per cent butanol, 10 per cent ethanol, and 50 per cent water, described in the original paper.

Fermentation.—A culture of the yeast *Saccharomyces bayanus* N.R.R.L.‡ No. 966) was used for fermenting glucose and one of *Saccharomyces carlsbergensis* (N.R.R.L. No. 379) was employed for fermenting glucose and galactose. The methods of fermentation used for the two yeasts were similar to those employed by Wise and Appling (8) and by Auernheimer *et al.* (2) except that the fermentation time was lengthened to 48 hours and the flasks were not shaken continuously.

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‡ Northern Regional Research Laboratory.

TABLE 1.—*Analyses of solutions of single sugars and synthetic mixtures of sugars by copper reduction and selective fermentation**

SUGAR	SINGLE SUGARS			MIXTURES OF TWO SUGARS				MIXTURE OF THREE SUGARS	
	GLUCOSE	GALACTOSE	RAMNOSE	GLUCOSE + GALACTOSE	GLUCOSE + RAMNOSE	GALACTOSE + RAMNOSE	GLUCOSE + GALACTOSE + RAMNOSE	GLUCOSE + GALACTOSE + RAMNOSE	GLUCOSE + GALACTOSE + RAMNOSE
Before Fermentation									
Mg. Sugar/aliqu. ^b	55.20	54.76	50.00	22.08 + 21.91	22.08 + 20.00	21.91 + 20.00	16.56 + 16.43 + 15.00		
Found	16.65	14.94	14.33	12.90	11.91	12.55	14.35		
MI. Thio. { Calc.	16.69	15.11	14.33	13.13	12.17	12.84	14.39		
% Recovery	99.8	98.9	100.0	98.3	97.9	97.8	99.7		
After Fermentation with Yeast N.R.R.L. No. 966 to Remove Glucose Only									
Mg. Sugar/aliqu. ^b	16.56	16.43	15.00	16.55 + 16.43	16.55 + 15.00	16.43 + 15.00	9.94 + 9.86 + 9.00		
Found	0.00	4.79	4.68	4.70	4.50	9.00	5.60		
MI. Thio. { Calc.	0.00	4.69	4.49	4.70	4.48	9.19	5.66		
% Recovery	—	102.1	104.2	100.0	100.2	98.0	98.9		
After Fermentation with Yeast N.R.R.L. No. 379 to Remove Glucose and/or Galactose									
Mg. Sugar/aliqu. ^b	16.56	16.43	15.00	16.55 + 16.43	16.55 + 15.00	16.43 + 15.00	9.94 + 9.86 + 9.00		
Found	0.00	0.00	4.60	0.00	4.33	4.38	2.68		
MI. Thio. { Calc.	0.00	0.00	4.49	0.00	4.48	4.48	2.74		
% Recovery	—	—	102.5	—	96.7	97.8	98.0		

* Duplicate stock solutions of the single sugars were made to volume. Aliquots of these solutions were mixed to give the sugar mixtures. All analyses were then made in duplicate.

^b Mg. sugar/aliquot = weight of the anhydrous sugar used in the final copper reduction; equivalent to the calculated ml. of thiosulfate.

PROCEDURE

Prepare a filter paper chromatogram of the solution to be analyzed. Determine the identity of the sugars present from the position of the spots. Dilute an aliquot of the original solution in the same manner as was done with the fermented samples. Using Schoorl's method, analyze an aliquot of this solution for total reducing sugars. Record the volume of 0.1 *N* thiosulfate required. If the chromatogram indicates that rhamnose is absent, but that either glucose or galactose or a mixture of these two is present, follow fermentation procedure A described below. If rhamnose is present in combination with either or both of the two sugars, follow procedure B. If the chromatogram indicates only rhamnose, fermentation is not necessary.

Fermentation Procedure A.—Ferment an aliquot of the solution with yeast N.R.R.L. No. 966 to remove glucose. Determine reducing materials on an aliquot of the fermented solution by Schoorl's method. Record the volume of 0.1 *N* thiosulfate. If the volume is zero, only glucose is present in the original solution. Calculate the weight of glucose present in the original solution by means of Schoorl's table from the volume of 0.1 *N* thiosulfate used before fermentation. If titration is required, the volume of 0.1 *N* thiosulfate is then equivalent to the galactose. From this volume, calculate the weight of glucose present in the original solution. If the two volumes are equal, only galactose is present in the original sample. Calculate the weight of galactose from either titration. If the two volumes are not equal, subtract the volume used by the solution after fermentation from the volume required for the total reducing sugars, to obtain the volume of 0.1 *N* thiosulfate equivalent to the glucose. Calculate the weight of glucose present in the original solution.

Fermentation Procedure B.—Ferment one aliquot of the original solution with yeast N.R.R.L. No. 966, and another with N.R.R.L. No. 379. The volume of 0.1 *N* thiosulfate after fermentation with yeast No. 379 is equivalent to the rhamnose in the aliquot taken for copper reduction, since both the glucose and/or galactose would be destroyed. Calculate the weight of rhamnose present in the original solution. The volume of 0.1 *N* thiosulfate after fermentation with yeast No. 966 is equivalent to the rhamnose and/or galactose, since only glucose would be destroyed. The difference between the volumes of 0.1 *N* thiosulfate required after fermentation with yeast No. 966 and No. 379 is equivalent to the galactose present. Calculate the weight of galactose present in the original solution. Subtract the volume of 0.1 *N* thiosulfate required after fermentation with yeast N.R.R.L. No. 966 from the volume of 0.1 *N* thiosulfate used in the aliquot analyzed before fermentation to obtain the volume of 0.1 *N* thiosulfate equivalent to the glucose. Calculate the weight of glucose in the original solution.

RESULTS

Table 1 shows the results obtained by using the above-described procedure with pure sugars, and with the several possible combinations of these sugars. To simplify the presentation of the data, the values are reported as ml. of 0.1 *N* thiosulfate rather than as mg. of sugar, since it is impossible to evaluate recoveries for any one step of the over-all procedure without incorporating any errors present in all the other steps. The experimental titres for a given weight of sugar are compared with the calculated titres. Percentage recovery is the ratio of ml. of thiosulfate found to the calculated volume of thiosulfate. Examples of an application of the method to the analysis of rutin are given in Table 2.

TABLE 2.—*Recoveries of Quercetin and Sugars from Rutin*

WEIGHT OF RUTIN	H ₂ SO ₄		TIME OF HYDROLYSIS	RECOVERY OF THEORETICAL		
	CONC.	VOL.		QUERCETIN ^a	RHAMNOSE	GLUCOSE
g.	per cent	ml.	hrs.	per cent	per cent	per cent
0.5000	2.5	25	2	100.3	95.5	96.0
.5000	5.0	25	1	100.6	95.2	95.8

^a Quercetin was filtered on Gooch crucible after hydrolysis, washed with water, dried to constant weight at 110°C. and weighed.

DISCUSSION

As indicated by the data, synthetic mixtures of glucose, galactose, and rhamnose can be analyzed, with recoveries of 98 to 104 per cent; hydrolysates of a flavonol glycoside can be analyzed, with recoveries of approximately 96 per cent. The value 96 per cent is probably low because of destruction of sugar during the hydrolysis. By use of the qualitative filter paper chromatogram, the amount of fermentation and chemical work required is reduced to the minimum. Application of the technique to other mixtures is being investigated.

SUMMARY

A method is presented for the analyses of mixtures of glucose, galactose and rhamnose in hydrolysates of flavonol glycosides. The sugar determinations are made by Schoorl's copper reduction method before and after fermentation by two yeasts capable of selective destruction of glucose and of glucose and galactose, respectively. Filter paper chromatography is used for qualitatively identifying the sugars.

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STUDIES ON COAL-TAR COLORS, VI*

D&C REDS NOS. 14, 15, 16, AND 31

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This paper represents the sixth in a series of papers (1, 2, 3, 4, 5) describing the preparation of purified samples of certifiable colors and the optical properties of solutions of those colors.

In this study the preparation, analysis, and optical properties in various solutions of 1-(2-carboxyphenylazo)-2-naphthol, the sodium, barium and calcium salts of which are certifiable as D&C Reds Nos. 14, 15, and 16 (6), respectively, and 1-phenylazo-3-carboxy-2-naphthol, the calcium salt of which is certifiable as D&C Red No. 31 (6), are described.

EXPERIMENTAL

Preparation of D&C Red No. 14.—Anthranilic acid and β -naphthol were recrystallized twice from water and benzene, respectively. Ten grams of anthranilic acid were dissolved in 300 ml. of water containing 20 ml. of conc. hydrochloric acid and the solution cooled to 5°C.; 5.5 grams of sodium nitrite previously dissolved in water was then added, and the mixture kept at 5°C. for one hour, stirring constantly. The excess nitrite was then destroyed with an excess of sulfamic acid. The solution of the resulting diazonium compound was added to a cold solution (5°C.) of 5 grams of sodium hydroxide, 15 grams of sodium carbonate, and 10.7 grams of β -naphthol in 500 ml. of water. The mixture was stirred continuously for one hour at a temperature of 5°C. and then was allowed to warm to room temperature. The product was collected in a Büchner funnel, and washed with two 50 ml. portions of water followed by two 50 ml. portions of ethyl alcohol and, finally, by several small portions of ether. The dye was dried thoroughly on a steam bath; then pulverized in a mortar and dried overnight at 135°C. Yield—18 grams.

Preparation of 1-(2-carboxyphenylazo)-2-naphthol (color acid of D&C Red No. 14).—Ten grams of the D&C Red No. 14 prepared above was suspended in 100 ml. of (1+5) HCl and the suspension warmed on a steam bath for 30 minutes. After cooling, the product was collected in a Büchner funnel and then recrystallized three times from glacial acetic acid.

Analysis:

Calculated: N, 9.59%

Found: N, 9.60%

Preparation of D&C Red No. 15.—A saturated solution of the sodium

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salt in boiling water was prepared and filtered, the filtrate heated to boiling and the barium salt precipitated by the addition of a slight excess of 10 per cent barium chloride solution. The product was collected in a Büchner funnel and washed with three 50 ml. portions of hot water, then with two 50 ml. portions of 95 per cent alcohol and, finally, with several small portions of ether. The material was dried on a steam bath, pulverized, and then dried for several hours at 135°C.

Analysis:

Calculated: Ba 19.1%

Found: Ba 18.9%

Titration with standard TiCl_3 soln: 99.7%

Preparation of the sodium salt of 1-phenylazo-3-carboxy-2-naphthol.—Aniline was purified by distillation. A commercial sample of 3-hydroxy-2-naphthoic acid was recrystallized three times from hot 50 per cent ethyl alcohol. This material melted at 221°C.; the reported value is 216°C. (7). Recrystallization of a small portion of the material from 95 per cent ethyl alcohol did not alter the melting point.

Ten grams of aniline was dissolved in 300 ml. of water containing 25 ml. of conc. hydrochloric acid and the solution cooled to 5°C. While stirring mechanically, 50. ml of a cold (5°C.) 16 per cent sodium nitrite solution was added and the solution held at 5°C. for one hour; then the excess nitrous acid was destroyed with sulfamic acid. The solution of the resulting diazonium compound was added to a cold (5°C.) solution of 20.5 grams of 3-hydroxy-2-naphthoic acid, 15 grams of sodium hydroxide and 15 grams of sodium carbonate, dissolved in 500 ml. of water. The mixture was stirred continuously for 30 minutes at 5°C., and then allowed to warm to room temperature. The product was collected on a Büchner funnel, and the material on the filter was washed successively with about 250 ml. of ca 0.1 *N* sodium hydroxide, about 200 ml. of distilled water, three 50 ml. portions of ethyl alcohol, and two 25 ml. portions of ether.

Preparation of 1-phenylazo-3-carboxy-2-naphthol.—The product obtained above was converted to the color acid and recrystallized three times from glacial acetic acid in the manner described for the preparation of 1-(2-carboxyphenylazo)-2-naphthol. The product crystallized in long, dark, red needles, m.p. 233°C. The melting point given in the Colour Index (8) is 232°C.

Analysis:

Calculated: N, 9.58%

Found: N, 9.60%

Five grams of the purified color acid was dissolved in about 300 ml. of hot ethyl alcohol and added slowly with rapid stirring to 500 ml. of hot water containing 10 grams of calcium acetate. After all of the color acid solution had been added, the resulting suspension was cooled to room

temperature and filtered on a Büchner funnel. The product remaining on the filter was resuspended in 500 ml. of distilled water, filtered on a Büchner funnel, and washed on the filter with three 50 ml. portions of ethyl alcohol and two 25 ml. portions of ether. The product was dried on a steam bath, pulverized in a mortar and dried at 135°C.

Analysis:

Calculated: N, 9.0 % Ca, 6.44 %
 Found: N, 9.01% Ca, 6.39%

SPECTROPHOTOMETRIC DATA

All spectrophotometric measurements were made with a General Electric recording spectrophotometer utilizing a wave length band of 8 m μ . Matched 1 cm. pyrex cells were used, and all solutions were allowed to come to room temperature before being made to volume. The pH values of the solutions were measured with a glass electrode pH meter in the range of pH 1.0 to 11.0, and the other values estimated. (Since the solvent in all cases was 50% alcohol it is to be understood that the term pH, as used here, is the apparent value.)

(a) *1-(2-carboxyphenylazo)-2-naphthol*:

(1) A 13.80 mg. sample of the purified color, weighed on a semimicro balance, was dissolved in exactly 500 ml. of 95% alcohol. Fifty ml. aliquots of this solution were diluted to 100 ml. with aqueous solutions of acid, alkali, and buffers, and the pH values of the solutions determined. The spectrophotometric data obtained from these solutions are shown in Figure 1.

(2) A 14.78 mg. sample of the purified color was dissolved in exactly 500 ml. of 95% alcohol. Aliquot portions of this solution were then diluted with water containing about 50 grams of ammonium acetate per liter, and the absorption spectra determined. The results are shown in Table 1 and Figure 2.

(b) *1-phenylazo-3-carboxy-2-naphthol*:

(1) Solutions containing 17.70 mg. of the purified color per liter were prepared as described under a(1). The spectrophotometric data obtained from these solutions are shown in Figure 3.

(2) Solutions of the purified color were prepared as described under a(2), except that the final dilutions were made with water containing 10 ml. of conc. hydrochloric acid per liter, and the absorption spectra determined. The results are shown in Table 2 and Figure 4.

DISCUSSION

The absorption curve of 1-(2-carboxyphenylazo)-2-naphthol in (1+1) alcohol-water solution is little changed with varying pH values between pH 1 and 13. At pH values between 7.0 and ca 12.0 there is practically no change in the curve. In this pH range the wave length of maximum ab-

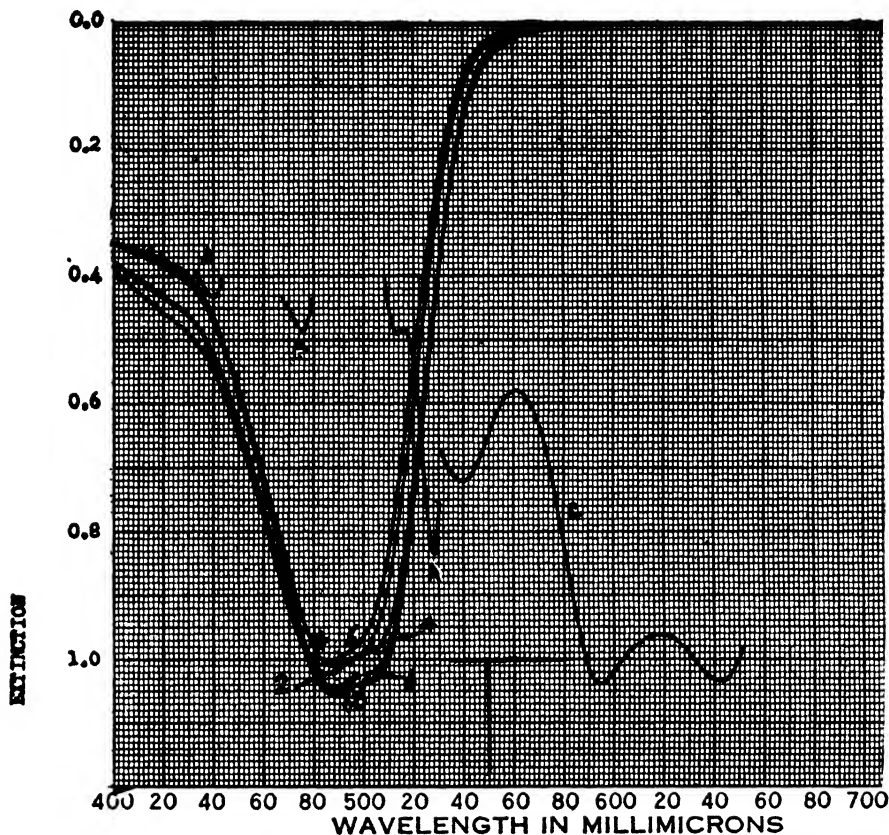


FIG. 1.—Absorption curves of D&C Red No. 14 in (1+1) alcohol-water solution at various pH levels (conc. 13.80 mg. per liter).

Curve 1—pH 7.1

Curve 4—pH 13.0

Curve 2—pH 4.7

Curve 5—pH 1.2

Curve 3—pH 8.2

Curve 6—pH 12.3

pH for curves 4 and 6 calculated, others measured with pH meter.

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$).

C = Signal Lunar White Glass-H-6946236.

sorption is $488 m\mu \pm 2 m\mu$. Solutions of this color at concentrations between 3.70 and 14.78 mg./liter were found to follow Beer's law (with a maximum deviation of 0.8% and an average deviation of 0.5%). The average extinction per milligram per liter in (1+1) alcohol-water solution at a pH between 7.0 and 12.0 and at 488 $m\mu$ was found to be 0.0770. This figure is based on the results of four determinations. Solutions stored at room temperature for 24 hours gave curves identical with those of freshly prepared solutions.

The absorption curve of 1-phenylazo-3-carboxy-2-naphthol in (1+1) alcohol-water solution changes in shape, wave length of maximum ab-

TABLE 1.—*Extinction values of purified 1-(2-carboxyphenylase)-2-naphthol dissolved in 50% alcohol*

CURVE NO.	CONCENTRATION MG./LITER	$E_{440\text{ m}\mu}$	$E_{440\text{ m}\mu}$
			CONCENTRATION
1	14.78	1.133	0.0767
2	11.09	0.849	0.0766
3	7.39	0.568	0.0769
4	3.70	0.287	0.0776
Average			0.0770

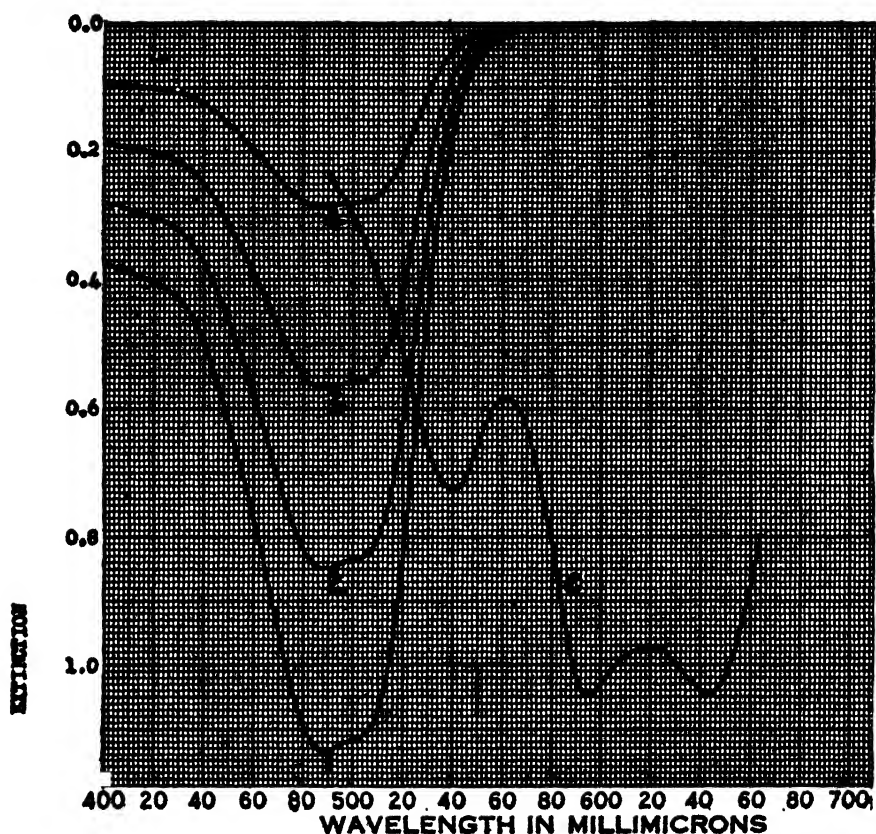


FIG. 2.—Absorption curves of D&C Red No. 14 in (1+1) alcohol-water solution buffered with ammonium acetate.

Curve 1—14.78 mg. per liter

Curve 2—11.09 mg. per liter

Curve 3—7.39 mg. per liter

Curve 4—3.70 mg. per liter

Cells—1 cm.

C—Signal Lunar White Glass-H-6946236.

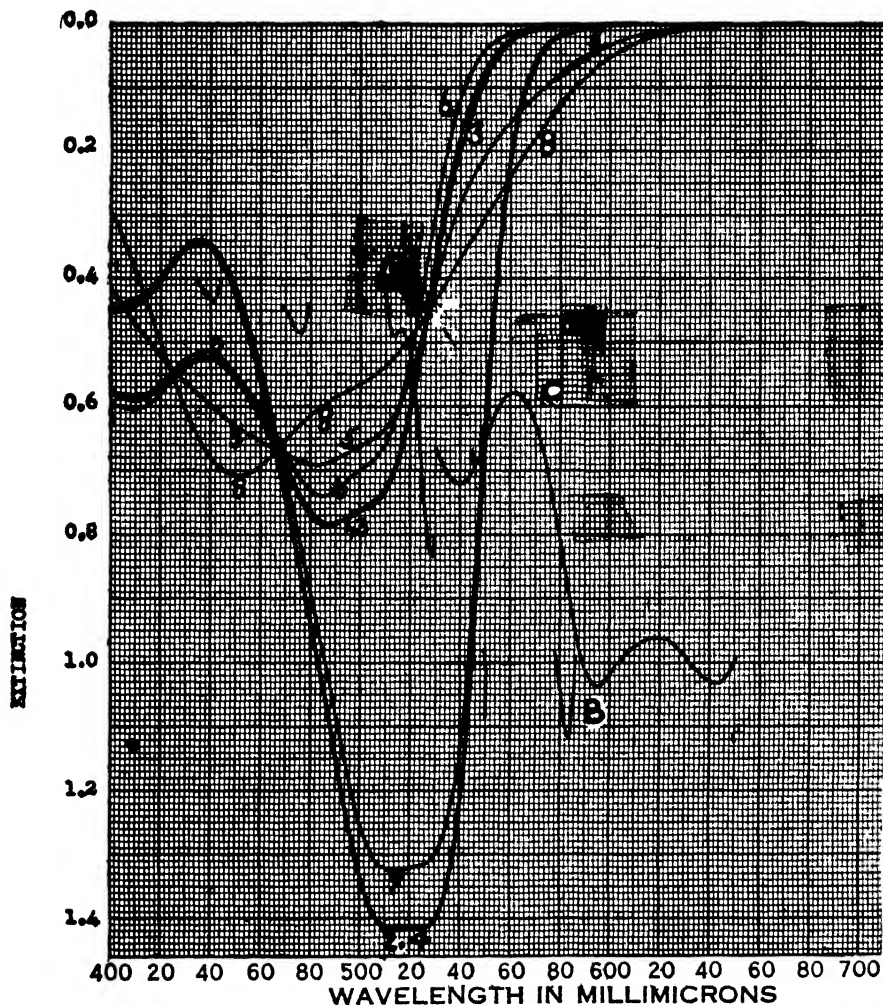


FIG. 3.—Absorption curves of D&C Red No. 31 in (1+1) alcohol-water solution at various pH levels (conc. 17.70 mg. per liter).

Curve 1—pH 6.7

Curve 5—pH 13.0

Curve 2—pH 3.9

Curve 6—pH 8.9

Curve 3—pH 6.9

Curve 7—pH 4.8

Curve 4—pH 1.2

Curve 8—pH 14.0

pH for curves 5 and 8 calculated, others measured with pH meter.

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$).

B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 $m\mu$).

C = Signal Lunar White Glass-H-6946236.

sorption, and unit extinction with varying values above pH 4.0. At pH values between 1.0 and 4.0 the curve is unchanged with variations in pH. Solutions of this color should, therefore, be adjusted to pH values be-

TABLE 2.—*Extinction values of purified 1-phenylazo-3-carboxy-2-naphthol dissolved in 50% alcohol*

CURVE NO.	CONCENTRATION MG./LITER	$E_{512} \text{ m}\mu$	$E_{512} \text{ m}\mu$
			CONCENTRATION
1	3.31	0.268	0.0810
2	6.62	0.535	0.0808
3	13.23	1.062	0.0803
Average			0.0807

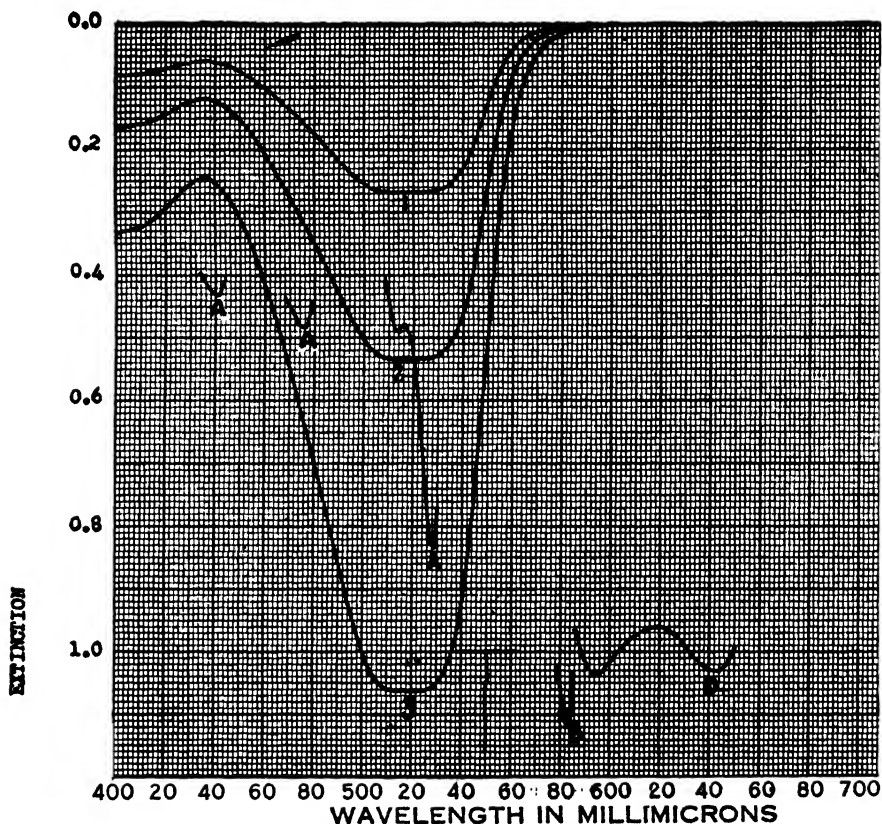


FIG. 4.—Absorption curves of D&C Red No. 31 in (1+1) alcohol-water solution adjusted to pH 2.5.

Curve 1—3.31 mg. per liter

Curve 2—6.62 mg. per liter

Curve 3—13.23 mg. per liter

Cells—1 cm.

A—Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $\text{m}\mu$).B—Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 $\text{m}\mu$).

C—Signal Lunar White Glass-H-6946236.

tween 1.0 and 4.0 before spectrophotometric measurements are made. The wave length of maximum absorption is $518\text{m}\mu \pm 6\text{m}\mu$. An unusual characteristic of curves of this color in acid solution is the broad, flat peak, there being practically no difference in the absorption between 512 and 524 $\text{m}\mu$. Solutions of this color at concentrations between 3.31 and 13.23 mg./liter were found to follow Beer's law (with a maximum deviation of 0.5% and an average deviation of 0.3%). The average extinction per milligram per liter in (1+1) alcohol-water solution at a pH between 1.0 and 4.0 and at 518 $\text{m}\mu$ was found to be 0.0807. This figure is based on the results of three determinations. Solutions stored for 24 hours at room temperature gave curves identical with those of freshly prepared solutions.

APPLICATION TO COMMERCIAL SAMPLES

Two commercial samples each of D&C Red No. 15, Barium Lake; and D&C Red No. 31, Calcium Lake, were analyzed spectrophotometrically. The samples were dissolved by refluxing with 250 ml. of 95% alcohol containing one ml. of conc. hydrochloric acid, cooling and diluting to exactly 500 ml. with 95% alcohol. Aliquots of the D&C Red No. 15, Lake, were diluted with equal volumes of water containing 50 grams of sodium acetate per liter. Aliquots of the solutions of D&C Red No. 31 were diluted with water containing 10 ml. of conc. hydrochloric acid per liter. The results of spectrophotometric examination of the resulting solutions are shown in Table 3.

TABLE 3.—*Analysis of commercial samples*

D&C RED NO. 15, BARIUM LAKE			D&C RED NO. 31, CALCIUM LAKE		
	DYE SPECTROPHOTO- METRICALLY	DYE BY TITRATION WITH TiCl_3		DYE SPECTROPHOTO- METRICALLY	DYE BY TITRATION WITH TiCl_3
	per cent	per cent		per cent	per cent
Sample No. 1	15.5	15.8	Sample No. 1	44.7	44.0
Sample No. 2	84.5	85.3	Sample No. 2	85.5	84.5

SUMMARY

Purified samples of D&C Red No. 14, 15 and their color acid 1-(2-carboxyphenylazo)-2-naphthol, and D&C Red No. 31 and its color acid (1-phenylazo-3-carboxy-2-naphthol), were prepared and studied spectrophotometrically.

The curve obtained from 1-(2-carboxyphenylazo)-2-naphthol in (1+1) alcohol-water solution adjusted to pH values between 7.0 and 12.0 is unchanged with changes in pH. At pH values below 7.0 and above 12.0 the curve changes slightly in regard to wave length of maximum absorption and general shape, but changes considerably in extinction. At pH values between 7.0 and 12.0 the wave length of maximum absorption is 488

$m\mu \pm 2 m\mu$. Beer's law is shown to be applicable to solutions containing 3.7 to 14.8 mg. of dye per liter. The average extinction per mg. per liter is 0.0770 ± 0.006 at 488 $m\mu$.

The curve obtained from 1-phenylazo-3-carboxy-2-naphthol in (1+1) alcohol-water solution adjusted to pH values between 1.0 and 4.0 is unchanged with changes in pH. At pH values above 4.0 the curve changes in regard to wave length of maximum absorption, extinction, and general shape of the curve. At pH values between 1.0 and 4.0 the wave length of maximum absorption is $518 m\mu \pm 6 m\mu$, there being practically no difference in absorption between 512 and 524 $m\mu$. Beer's law is shown to be applicable to solutions containing 3.31 to 13.23 mg. of dye per liter. The average extinction per mg. per liter is 0.087 ± 0.003 at 518 $m\mu$.

These data have been applied to the determination of "pure color" in commercial samples of D&C Red No. 15, Barium Lake, and to D&C Red No. 31, Calcium Lake.

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STUDIES ON COAL-TAR COLORS, VII*

D&C RED NO. 34

By KENNETH A. FREEMAN and CHARLES GRAICHEN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington 25, D.C.)

This is the seventh in a series of papers† dealing with the preparation, analysis, and optical properties of solutions of certifiable coal-tar colors.

In this paper, the results obtained in the investigation of 4-(1-sulfo-2-

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 10-12, 1949.

† For references to previous papers see No. VI. *This Journal*, p. 718.

naphthylazo)-3-hydroxy-2-naphthoic acid, the calcium salt of which is certifiable as D&C Red No. 34,¹ are described.

EXPERIMENTAL

Purification of intermediates

Tobias acid (2-naphthylamine-1-sulfonic acid).—A sample of commercial Tobias acid was recrystallized three times from warm (1+1) alcohol-water solution. The temperature was not allowed to exceed 70°C. in order to avoid possible rearrangement of the sulfonic acid group.

Analysis:

Calculated: S, 14.30%

Found: S, 14.34%

3-Hydroxy-2-naphthoic acid.—A sample of the commercial material was recrystallized three times from hot (1+1) alcohol-water solution. The melting point of the purified material (221°C.) was not changed by a fourth recrystallization from hot 95% alcohol.

Preparation of D&C Red No. 34.—Thirty grams of Tobias acid was dissolved in 950 ml. of distilled water containing 7.0 grams of sodium hydroxide. Forty-seven ml. of conc. hydrochloric acid was added slowly, with vigorous stirring, to form a finely divided suspension of the acid. A 10% sodium nitrite solution cooled to below 5°C. was added to the cold suspension (5°C.) and the mixture stirred for one hour at 5°C. The excess nitrite was then destroyed with excess sulfamic acid. The suspension of the resulting diazonium compound was immediately added to a cold (5°C.) solution of 27 grams of 3-hydroxy-2-naphthoic acid, 12.9 grams of sodium hydroxide and 29 grams of sodium carbonate in one liter of distilled water. The mixture was stirred continuously for one hour at a temperature of 5°C. and then was allowed to warm to room temperature. The precipitated product was collected on a Büchner funnel and recrystallized from distilled water. The dye was again collected on the Büchner funnel and washed with three 100-ml portions of ethyl alcohol, followed by several small portions of ether.

A part of the dye was suspended in water containing twice the amount of calcium chloride calculated to give the calcium salt of the dye, and boiled for 30 minutes. The material was filtered while hot and was washed successively with water, alcohol, and ether. The ether was evaporated on a steam bath and the dye pulverized in a mortar.

The dried color absorbs moisture so rapidly from the air that accurate weighing is impractical. The material was therefore spread out on a large watch-glass in a dust-free atmosphere until the moisture content had reached equilibrium. The results of the analyses are shown in Table 1.

¹ Service and Regulatory Announcements, F.D.C. No. 3, Food and Drug Administration.

There is a difference in water content of 1.66% between the dye dried at atmospheric pressure and at 2 mm. pressure. Since this is equivalent to 0.48 mols of water, it is probable that one-half molecule of water of crystallization remains in the product dried at 135°C. at atmospheric pressure.

SPECTROPHOTOMETRIC DATA

All spectrophotometric measurements were made with a General Electric recording spectrophotometer, utilizing a wave length band of 8 m μ . Matched 1 cm. Pyrex cells were used and all solutions were allowed to come to room temperature before being made to volume.

(a) A 15.17 mg. sample (corrected to the anhydrous color) of the puri-

TABLE 1.—*Analysis of purified D&C Red No. 34*

DETERMINATION	CALCULATED FOR PURE COLOR	FOUND	PURE DYE BY CALCU- LATION
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Nitrogen	6.08	5.42	89.1
Sulfur	6.96	6.20	89.1
Calcium	8.70	7.68	88.2
Titration with 0.1 N TiCl ₃	—	—	88.4
Volatile matter at 135°C. and atm. press.	—	8.94	—
Volatile matter at 138°C. and 2 mm. press.	—	10.60	—
Titration of the dried material with 0.1 N TiCl ₃	—	—	99.3*

* This figure is probably low because of the absorption of moisture by the sample while weighing.

fied color was weighed on a semi-micro balance and dissolved by refluxing for 15 minutes in 250 ml. of 95% alcohol containing 1 ml. of conc. hydrochloric acid. The solution was cooled to room temperature and diluted to exactly 500 ml. with 95% alcohol. 50 ml. aliquots of this solution were diluted to 100 ml. with aqueous solutions of acid, alkali, or buffers. The pH values of these solutions were determined with a glass electrode pH meter in the range of pH 1.0 to 11.0, and the other values estimated. (Since it is doubtful whether the indicated pH values of alcoholic solutions are true values, it is to be understood that the term pH, as used here is the apparent value.) The spectrophotometric data obtained from these solutions are shown in Figure 1.

(b) A 17.09 mg. sample (corrected to the anhydrous color) of the purified color was dissolved in exactly 500 ml. of 95% alcohol as described in (a). Aliquot portions of this solution were then diluted with water containing 10 ml. of conc. hydrochloric acid per liter. The spectrophotometric data obtained from these solutions are shown in Table 2 and Figure 2.

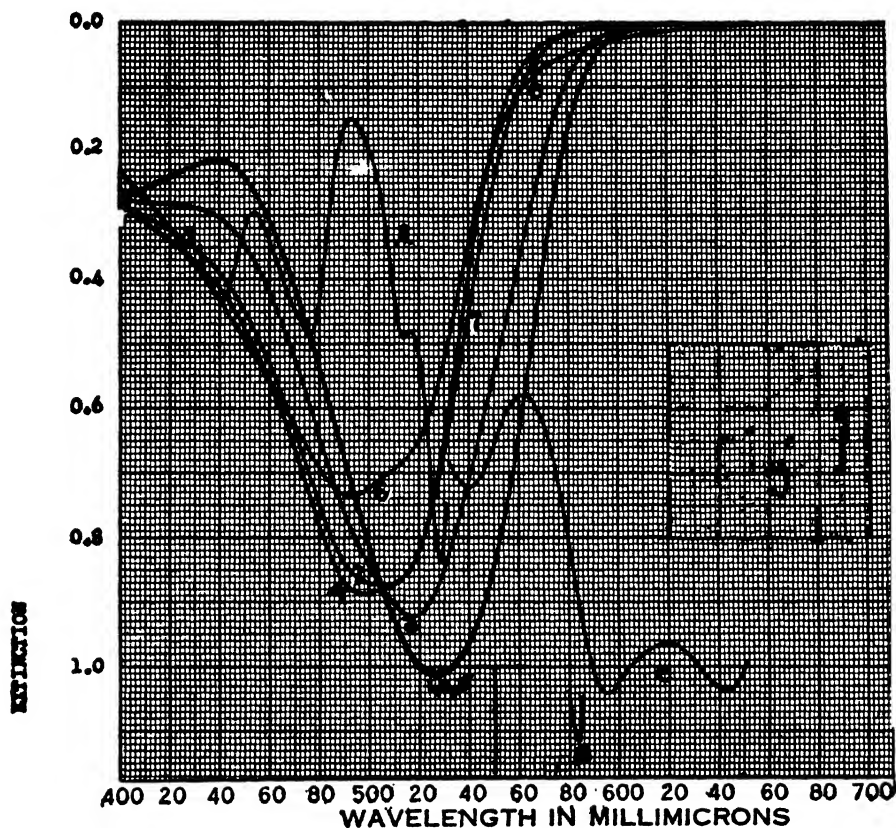


FIG. 1.—Absorption curves of D&C Red No. 34 in alcohol-water solution of various pH levels (conc. 15.17 mg. per liter).

Curve 1—pH 2.2

Curve 2—pH 3.4

Curve 3—pH 5.8

Curve 4—pH 13.0

Curve 5—pH 1.1

Curve 6—pH 14.0

Curve 7—pH 8.4

A—Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 $m\mu$).

B—Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 $m\mu$).

C—Signal Lunar White Glass-H-6946236.

DISCUSSION

The absorption curve of D&C Red No. 34 in (1+1) alcohol-water solution changes in shape, wave length of maximum absorption, and unit extinction, with varying values above pH 3.5. At pH values between 1.0 and 3.5 the curve is unchanged with variations in pH. Solutions of this color should therefore be adjusted to pH values between 1.0 and 3.5 before spectrophotometric measurements are made. The wave length of maximum absorption is $528 m\mu \pm 2 m\mu$.

Solutions of this color at concentrations between 6.8 and 17 mg./liter

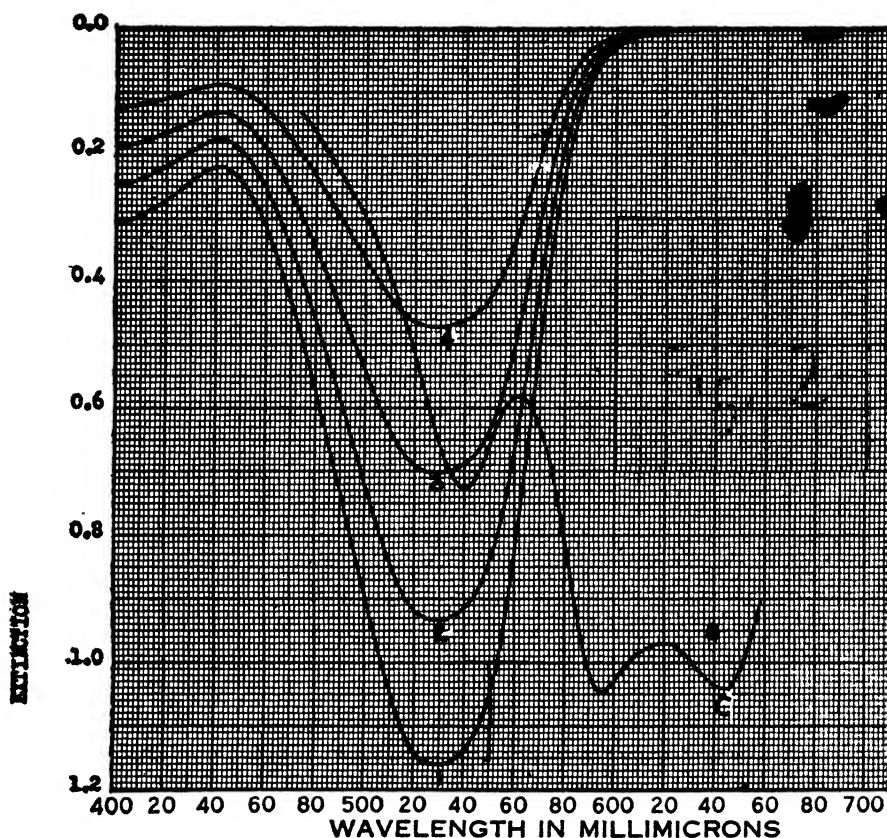


FIG. 2.—Absorption curves of D&C Red No. 34 in (1+1) alcohol-water adjusted to pH 2.6.

Curve 1—17.09 mg./liter

Curve 2—13.68 mg./liter

Curve 3—10.25 mg./liter

Curve 4—6.84 mg./liter

Cells—1 cm.

C—Signal Lunar White Glass-H-6946236.

All concentrations calculated as anhydrous colors.

TABLE 2.—Extinction values of purified D&C Red No. 34 dissolved in 50% alcohol

CURVE NO.	CONCENTRATION mg./LITER	E _{555 mμ}	E _{555 mμ}
			CONCENTRATION
1	17.09	1.160	0.0679
2	13.68	0.933	0.0682
3	10.25	0.703	0.0686
4	6.84	0.471	0.0688
Average			0.0684

follow Beer's law (with a maximum deviation of 0.7% and an average deviation of 0.5%). The average extinction per milligram per liter in (1+1) alcohol-water solution at a *pH* between 1.0 and 3.5, and at 528 $m\mu$, was found to be 0.0684. This figure is based on the results of four determinations. Solutions stored for 24 hours at room temperature gave curves identical with those of freshly prepared solutions.

APPLICATION TO COMMERCIAL SAMPLES

Two commercial samples of D&C Red No. 34, Calcium Lake, were analyzed spectrophotometrically following the procedure described for the standard sample. The data are shown in Table 3.

TABLE 3.—*Analysis of commercial samples of
D&C Red No. 34, Calcium Lake*

	DYE SPECTRO- PHOTOMETRICALLY	DYE BY TITRATION WITH $TiCl_3$
	<i>per cent</i>	<i>per cent</i>
Sample No. 1	60.9	61.5
Sample No. 2	69.5	70.5

SUMMARY

Purified D&C Red No. 34 was prepared and studied spectrophotometrically. The curve obtained from the color in (1+1) alcohol-water solution, adjusted to *pH* values between 1.0 and 3.5, is unchanged with changes in *pH*. At *pH* values above 3.5 the curve changes in regard to wave length of maximum absorption, extinction, and general shape. At *pH* values between 1.0 and 3.5 the wave length of maximum absorption is 528 $m\mu$ +2 $m\mu$. Beer's law is shown to be applicable to the solutions containing 6.84 to 17.09 mg. of dye per liter. The average extinction per mg. per liter is 0.0684 ± 0.004 at 528 $m\mu$. These data have been applied to the determination of "pure color" in commercial samples of D&C Red No. 34, Calcium Lake.

WATER-INSOLUBLE FATTY ACIDS AND BUTYRIC ACID IN CREAM AND BUTTER*

By FRED HILLIG, H. A. LEPPER, and W. I. PATTERSON
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INTRODUCTION

Spoilage resulting from improper practices in the production of cream intended for use in the manufacture of butter has long been a problem,

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 10-12, 1949.

† W. B. White, Chief.

not only for the manufacturer, but also for the officials charged with the enforcement of food laws. One of the corrective steps taken by industry has been the inauguration of widespread cream improvement programs. While considerable improvement has resulted from these programs, decomposed cream, unfit for human consumption, is still being used in the manufacture of butter.

The time required for a given raw cream to become decomposed will depend chiefly on the temperature at which it is held unrefrigerated but decomposition is progressive in every case. The chemical break-down which occurs during these progressive changes seems to center mainly in three cream constituents. Lactose is degraded into smaller molecules such as lactic acid, acetic acid, and sometimes (particularly in the later stages) propionic acid and butyric acid. Protein is hydrolyzed to polypeptides, and to some extent to the individual amino acids. Fat is hydrolyzed to its constituent fatty acids and glycerol; and the unesterified water-insoluble fatty acids (WIA)¹ can be determined. In any particular case any one of these types of chemical changes may or may not occur; *i.e.*, cream may decompose into other organoleptically disagreeable substances without a significant increase in WIA or butyric acid.

STUDIES ON PROGRESSIVE DECOMPOSITION

Progressive decomposition studies similar to those reported in a previous paper,² were conducted on a series of creams produced on farms in the vicinity of Cincinnati during the summer of 1947. It was originally planned to use the cream from one producer only; but owing to the fact that most of the producers in the neighborhood of Cincinnati are small ones, it was not possible to obtain from any single source the 10 gallons of cream required for the experiment. Arrangements were therefore made with several producers along one route to hold the cream separated from combined morning and evening milkings. These creams were then collected on the following morning and composited in a 10 gallon cream can.

On receipt at the laboratory it was found that the creams had developed some acidity (0.25 to 0.37 per cent titrable acidity as lactic). Obviously authentic cream with no developed acidity would have been preferable as the starting material for progressive decomposition studies. Although the creams were not under observation from the beginning, and souring had started when the experiments were begun, valuable information was obtained by allowing these creams to progressively decompose. They were well mixed and about 18 pounds were removed from the can. After samples for chemical analysis were taken the balance of the 18 pound portion was pasteurized at 150°F for 30 minutes, cooled, and churned in an electrically driven churn of 12 gallon capacity. The remainder of the cream in each 10 gallon can was allowed to stand on the receiving floor of the creamery

¹ *This Journal*, 30, 575 (1947).

² *This Journal*, 31, 750 (1948).

for periods up to 11 days. The creams were stirred daily, and samples were taken for analysis at three intervals several days apart. At each sampling a portion was neutralized, pasteurized, and churned. Data obtained on the first cream studied are given in Table 1.

TABLE 1.—*Changes in cream during progressive decomposition*
Temperature range 65–91°F. Relative humidity 73–99%

DETERMINATIONS	CREAM				BUTTER			
	AGE IN DAYS				AGE IN DAYS			
	1	4	8	11	1	4	8	11
Titration Acidity % as Lactic	0.25	0.76	1.59	1.62	—	—	—	—
Butyric Acid ¹ mg/100 g Fat	None	16.4	18.7	12.8	None	2.4	3.0	6.6
Propionic Acid mg/100 g Fat	None	None	None	5.4	None	None	None	1.5
Fat, Per Cent	31.0	31.0	31.0	31.0	87.5	83.9	84.9	82.7
Acidity of Fat ml 0.1 N/100 g	—	—	—	—	13	11	16	11
Lactic Acid, Per Cent	0.222	0.358	0.977	0.727	—	—	—	—
Lactose, Per Cent	1.33	0.85	0.00	0.00	—	—	—	—
WIA mg/100 g Fat	234	657	934	1052	225	659	952	1084
WIA Buttermilk mg/100 g	—	—	—	—	14	29	32	27
Odor and Flavor	Normal	Moldy	Moldy Cheesy	Cheesy Putrid	Rancid taste after 4 days at 40°F.	Decided objection- able flavor	Cheesy	Cheesy Putrid

¹ *This Journal*, 28, 644 (1945).

The titrable acidities increased rapidly, but there was no increase in lactic acid after 8 days; the lactose had by then completely disappeared from the cream. Some butyric acid was found in the cream after 4, and also after 8 and 11 days, and some of this acid carried over into the butter. Propionic acid was found in the cream after 11 days of holding. It has been shown in a previous report² that the presence of determinable quantities of butyric acid (or propionic acid) in butter is associated with the presence of some decomposed cream in the vat mixture from which the butter was churned. Progressively larger quantities of WIA were found in the cream and butter after the 4, 8, and 11 days of holding. Two of the producers used mechanical separators and the other four, water separators.

In like manner a second composite can of cream was obtained from a different group of producers. The analytical data are given in Table 2.

Lactose did not completely disappear from this cream even after holding 9 days. An increase in WIA was found in the sample taken on the 6th day, at which time butyric and propionic acids had also appeared in the cream. Four of the seven producers used mechanical separators, and the other three, water separators.

TABLE 2.—*Changes in cream during progressive decomposition*
Temperature range 54–92°F. Relative humidity 68–100%

DETERMINATIONS	CREAM				BUTTER			
	AGE IN DAYS				AGE IN DAYS			
	1	3	6	9	1	3	6	9
Titration Acidity % as Lactic	0.30	0.70	1.20	1.80	—	—	—	—
Butyric Acid ¹ mg/100 g Fat	Trace	None	10.4	—	Trace	—	Trace	8.5
Propionic Acid mg/100 g Fat	Trace	Trace	7.4	—	Trace	—	Trace	None
Fat, Per Cent	37.0	36.0	36.6	37.2	83.7	87.6	85.0	87.3
Acidity of Fat ml 0.1 N/100 g	—	—	—	—	6	5	6	9
Lactic Acid, Per Cent	0.185	0.466	0.940	1.078	—	—	—	—
Lactose, Per Cent	2.43	1.99	1.24	0.41	—	—	—	—
WIA mg/100 g Fat	75	68	314	758	153	135	312	714
WIA Buttermilk mg/100 g	—	—	—	—	20	26	24	35
Odor and Flavor	Normal	Normal	Moldy Slight Cheesy	Moldy Cheesy Rancid	Normal	Normal	Slight Cheesy	Cheesy

¹ *This Journal*, 28, 644 (1945).

A third cream similarly obtained from a still different group of producers yielded, on a study of its progressive decomposition, the analytical data in Table 3.

At the end of 4 days holding, it was found that the cream had undergone considerable deep-seated chemical break-down, as is evidenced from the quantity of WIA found, and also by the appearance of butyric and propionic acids. The cream was not held beyond the 7th day, since its condition had so deteriorated that, it appeared nothing could be gained by carrying the experiment further. It is interesting to note that each of

the producers who furnished the cream for this experiment used water separators.

A progressive decomposition study was also conducted on a cream, freshly separated from about 100 gallons of sweet milk obtained from a single producer. The data are given in Table 4.

WIA in the butter (made as before in the churn of 12-gallon capacity) from this cream, at the start of the experiment, is well within the range

TABLE 3.—*Changes in cream during progressive decomposition*
Temperature range 60–101°F. Relative humidity 82–100%

DETERMINATIONS	CREAM			BUTTER		
	AGE IN DAYS			AGE IN DAYS		
	1	4	7	1	4	7
Titration Acidity % as Lactic	0.37	0.96	1.38	—	—	—
Butyric Acid ¹ mg/100 g Fat	None	26.0	103.6	—	2.8	2.8
Propionic Acid mg/100 g Fat	None	11.1	43.3	—	2.1	2.1
Fat, Per Cent	36.0	33.8	29.0	85.5	78.7	84.2
Acidity of Fat ml 0.1 N/100 g	—	—	—	11	26	15
Lactic Acid, Per Cent	0.253	0.466	0.680	—	—	—
Lactose, Per Cent	1.09	0.54	None	—	—	—
WIA mg/100 g Fat	237	1037	1711	235	970	1170
WIA Buttermilk mg/100 g	—	—	—	17	45	336
Odor and Flavor	Normal	Moldy	Moldy	Distinct objection- able taste	Cheesy Rancid	Putrid Rancid

¹ *This Journal*, 28, 644 (1945).

found for sweet cream butter² and it did not increase until after 8 days of holding the cream. The initial lactose closely approximated that normally found in machine-separated sweet cream. As the cream became more and more sour, the lactose never completely disappeared, as was the case in two of the other progressive decomposition experiments on multiple-source cream described above, where the initial quantity of lactose was smaller. Furthermore the quantity of lactic acid found in the original cream corresponded to that usually found in sweet cream (0 to 3

² *This Journal*, 31, 739 (1948).

milligrams per 100 grams). Butyric and propionic acids did not appear, other than in traces, in the cream until after 8 days.

STUDIES ON CREAM OF INDIVIDUAL PRODUCERS

Arrangements were made with each of six of the producers on one of the cream routes to furnish cream separated from the morning and evening milking of the preceding day. The analysis of these samples is given in Table 5.

TABLE 4.—*Changes in cream during progressive decomposition*
Temperature range 71–95°F. Relative humidity 86–100%

DETERMINATIONS	CREAM				BUTTER			
	AGE IN DAYS				AGE IN DAYS			
	1	3	8	11	1	3	8	11
Titration Acidity % as Lactic	0.12	0.66	1.09	1.54	—	—	—	—
Butyric Acid ¹ mg/100 g Fat	None	Trace	39.0	22.1	None	Trace	None	2.4
Propionic Acid mg/100 g Fat	None	Trace	36.8	12.3	None	Trace	None	None
Fat, Per Cent	28.0	27.0	26.0	27.0	84.5	81.9	69.4	75.7
Acidity of Fat ml 0.1 N/100 g	—	—	—	—	7	18	19	16
Lactic Acid, Per Cent	0.003	0.309	0.635	0.966	—	—	—	—
Lactose, Per Cent	2.75	2.35	1.63	0.86	—	—	—	—
WIA mg/100 g Fat	176	251	684	762	158	219	700	740
WIA Buttermilk mg/100 g	—	—	—	—	20	24	38	74
Odor and Flavor	Normal	Normal	Slight Cheesy Slight Rancid	Cheesy Putrid	Normal	Normal	Slight Cheesy	Cheesy Rancid

¹ *This Journal*, 28, 644 (1945).

There is a wide variation in the percentage of lactose in these creams. Samples 1, 4, 5, and 6, in which the cream was separated in a water separator, contained the lower quantities of lactose and, with one exception, contained higher quantities of WIA; samples 1, 4, and 6 contained somewhat more of these acids than has been found in authentic undecomposed cream.

In order to compare water and mechanically-separated cream the farm of producer No. 1 was revisited and arrangements made to obtain the

TABLE 5.—*Analysis of cream from individual producers*

PRODUCER NUMBER	TITRABLE ACIDITY	FAT	LACTOSE	WIA	SEPARATOR USED
	<i>Per cent Lactic</i>	<i>per cent</i>	<i>per cent</i>	<i>Mg/100 g Fat</i>	
1	0.11	44	0.36	234	Water
2	0.35	47	2.40	74	Mechanical
3	0.30	51	2.43	176	Mechanical
4	0.43	38	0.50	366	Water
5	0.45	38	0.88	163	Water
6	0.42	35	1.28	246	Water

evening milk as soon as possible after milking. A small hand separator was taken along and the cream was separated immediately after milking. Analysis was started immediately in order to stop any bacterial action, and then finished in the laboratory. The data are given in Table 6. This table also gives data obtained 4 weeks earlier on cream from the same farm which had been separated in a water separator. The samples were taken from the combined creams of the morning and evening milking in each instance.

The data are rather striking. Titrable acidities are the same. Lactose in the mechanically separated cream is normal for this type of separation, while the small quantity found in the cream obtained from the water separator indicates that most of the lactose was lost because of its solubility in the serum. WIA in the mechanically separated cream is well below the maximum found for sweet cream.

In a further study of cream from these producers, arrangements were made with the centralizer receiving these producers' creams to set aside the normal weekly deliveries made by each. These creams were not mixed with creams from other producers. Thus there was, in each delivery, cream from 1 to 7 days old. After samples of the cream were taken for analysis the balance was neutralized, pasteurized, and churned as before. Analyses of the creams and butters are given in Table 7.

All these creams were cheesy in taste and odor, demonstrating that a material quantity of decomposed cream was present. This is confirmed

TABLE 6.—*Comparative analysis of creams, water and mechanically separated*

	WATER SEPARATED	MECHANICALLY SEPARATED
Titration Acidity (per cent as Lactic)	0.11	0.10
Fat (per cent)	44.0	56.0
Lactose (per cent)	0.36	2.13
WIA (Mg/100 g fat)	234	82

TABLE 7.—*Analysis of weekly delivery of cream from individual producers*

PRODUCER NO.	CREAM					BUTTER				
	TITRABLE ACIDITY	BUTYRIC ¹ ACID	PROPIONIC ACID	FAT	WIA	FAT	ACIDITY OF FAT	BUTYRIC ¹ ACID	WIA	WIA IN BUTTERFAK
	Per cent as Lactic	mg/100 g	mg/100 g	per cent	mg/100 g Fat	per cent	ml 0.1 N/100 g	mg/100 g	mg/100 g Fat	mg/100 g
1	0.55	44.6	10.8	39.0	1440	83.4	33	6.5	1361	112
2	0.88	43.9	17.6	33.0	1229	80.1	25	4.5	1097	129
3	1.41	—	—	53.0	475	84.6	5	2.6	410	209
4	1.09	195.0	47.4	35.0	1399	—	—	—	—	—
5	1.00	81.9	31.7	38.0	1615	82.1	20	9.0	1288	256
6	1.43	61.0	17.8	35.0	1556	82.9	24	3.5	1517	89

¹ *This Journal*, 28, 644 (1945).

TABLE 8.—*Creams and butters churned therefrom obtained from individual producers*

SAMPLE NO.	CREAM					BUTTER					BUTTER MILK		COMMENT
	TITRABLE ACIDITY	FAT	BUTYRIC ¹ ACID	PROPI- ONIC ¹ ACID	WIA	ACTIVITY OF FAT	BUTYRIC ¹ ACID	PROPI- ONIC ¹ ACID	FAT	WIA	WTA		
	per cent as lactic	per cent	mg/100 g fat	mg/100 g fat	mg/100 g fat	ml 0.1 N per 100 g fat	mg/100 g fat	mg/100 g fat	per cent	mg/100 g fat	mg/100 g		
1	0.93	34.0	11.6	3.6	206	6	Trace	None	84.0	23		Sour cream.	
2	1.37	32.0	7.9	None	355	12	Trace	None	85.0	22		Cream had disagreeable taste; not identified.	
3	0.62	24.6	—	—	45	4	None	None	70.1	20		Sour cream.	
4	0.90	26.0	7.3	8.1	281	8	None	None	78.2	27		Sour cream.	
5	1.01	26.2	29.7	11.6	447	9	None	None	79.3	37		Cream slightly cheesy. Butter slightly cheesy	
6	0.71	35.0	None	None	—	5	None	None	84.0	23		Sour cream.	
7	1.52	42.4	22.4	5.0	802	13	5.4	None	83.6	55		Cream slightly cheesy. Fruity odor. Entire surface	
8	0.88	40.0	10.3	6.8	501	10	2.4	Trace	84.0	22		of can covered with heavy corrugated mold layer.	
9	0.59	39.0	12.2	None	404	12	None	None	82.8	34		Cream after pasteurization had a slight	
10	0.72	43.0	None	None	130	8	None	None	83.8	29		cheesy odor. Butter had disagreeable taste.	
11	1.44	34.0	144.4	9.0	1342	13	10.8	3.1	70.9	605		Sour cream.	
12	1.36	25.2	None	None	159	9	None	None	85.7	19		Cream was cheesy and putrid.	
13	1.39	22.0	Trace	Trace	82	7	None	None	85.8	21		Cream after pasteurization had a slight	
14	2.12	36.0	50.1	10.9	2402	20	2.5	None	82.6	463		yeasty odor.	
15	2.46	12.0	171.6	88.4	5983	9	3.0	2.3	85.3	487		Sour cream.	
16	0.62	45.0	28.2	None	405	46	28.6	None	82.4	61		Cream was cheesy and putrid.	
17	0.94	42.0	—	—	242	7	None	None	84.2	30		Cream slightly cheesy. Butter had disagree- able taste.	
18	0.73	32.0	19.8	6.6	697	10	None	None	84.0	46		Cream slightly cheesy. Butter slightly cheesy	
19	0.99	32.0	None	None	330	7	None	None	85.5	8		and had disagreeable taste.	
20	1.02	38.0	None	None	2418	29	None	None	80.4	525		Butter slightly cheesy and had disagreeable	
21	0.89	24.0	51.5	14.5	245	11	6.4	None	86.0	24		taste.	
22	0.65	56.0	18.7	5.4	654	27	6.4	None	73.9	34		Cream slightly cheesy.	
												Sour cream.	
												Butter slightly cheesy and had disagreeable	
												taste.	

¹ *This Journal*, 28, 844 (1945).

TABLE 9.—Analysis of survey samples of butter¹ (1945)

CHICAGO				ST. LOUIS				NEW ORLEANS				CINCINNATI			
SAMPLE NO.	WTA	BUTTERIC ²	MOLD	SAMPLE NO.	WTA	BUTTERIC ²	MOLD	SAMPLE NO.	WTA	MOLD	SAMPLE NO.	SAMPLE NO.	WTA	MOLD	SAMPLE NO.
	mg/100 g Fat	mg/100 g Fat			mg/100 g Fat	mg/100 g Fat			mg/100 g Fat				mg/100 g Fat		
1	29	—	2	1	81	—	0	1	21	6	1	1	98	0	1
2	39	—	18	2	99	—	0	2	29	8	2	2	104	0	2
3	45	—	16	3	106	—	0	3	54	10	3	3	116	6	3
4	51	—	2	4	124	—	6	4	63	10	4	4	150	44	4
5	53	—	2	5	129	—	8	5	65	6	5	5	150	92	5
6	54	—	0	6	138	—	0	6	73	0	6	6	181	0	6
7	56	—	0	7	146	—	16	7	94	0	7	7	197	45	7
8	63	—	24	8	151	—	4	8	104	8	8	8	210	75	8
9	66	—	0	9	154	—	79	9	108	8	9	9	249	97	9
10	66	—	0	10	160	—	44	10	119	26	10	10	254	0	10
11	75	—	0	11	161	—	0	11	134	14	11	11	266	91	11
12	89	—	0	12	161	—	0	12	149	18	12	12	266	92	12
13	101	—	0	13	165	—	48	13	190	54	13	13	269	15	13
14	109	—	0	14	171	—	0	14	218	62	14	14	298	89	14
15	114	—	42	15	174	—	60	15	223	18	15	15	314	70	15
16	116	—	52	16	191	—	0	16	226	22	16	16	330	93	16
17	118	—	0	17	198	—	87	17	244	26	17	17	425	93	17
18	119	—	0	18	203	—	85	18	269	54	18	18	441	66	18
19	119	—	4	19	211	—	56	19	278	32	19	19	453	93	19
20	121	—	2	20	226	—	74	20	280	10	20	20	465	90	20
21	133	—	40	21	235	—	56	21	285	34	21	21	598	83	21
22	133	—	29	22	244	—	84	22	288	30	22	22	649	82	22
23	136	—	34	23	251	—	40	23	294	42	23	23	2063	96	23
24	141	—	0	24	264	—	50	24	299	42	24	24	2341	82	24

TABLE 9—(continued)

CHICAGO				ST. LOUIS				NEW ORLEANS				CINCINNATI			
SAMPLE NO.	WIA	BUTYRIC ¹	MOLD	SAMPLE NO.	WIA	BUTYRIC ¹	MOLD	SAMPLE NO.	WIA	MOLD	SAMPLE NO.	WIA	MOLD	SAMPLE NO.	MOLD
	mg/100 g Fat	mg/100 g Fat			mg/100 g Fat	mg/100 g Fat			mg/100 g Fat			mg/100 g Fat			mg/100 g Fat
25	143	—	28	25	278	—	62	25	331	51	25	331	51	25	331
26	145	—	21	26	285	—	44	26	325	28	26	325	28	26	325
27	151	—	2	27	293	—	50	27	335	38	27	335	38	27	335
28	168	—	2	28	296	—	42	28	340	30	28	340	30	28	340
29	174	—	4	29	304	—	44	29	343	18	29	343	18	29	343
30	181	—	2	30	320	—	52	30	359	58	30	359	58	30	359
31	195	—	35	31	333	—	70	31	386	38	31	386	38	31	386
32	215	—	29	32	363	—	91	32	414	72	32	414	72	32	414
33	238	3	88	33	386	—	78	33	450	22	33	450	22	33	450
34	243	3	82	34	394	—	81	34	456	44	34	456	44	34	456
35	464	11	22	35	414	—	72	35	456	22	35	456	22	35	456
36	501	23	58	36	541	9	22	36	505	20	36	505	20	36	505
37	599	13	26	37	589	—	64	37	519	56	37	519	56	37	519
38	733	11	24	38	593	15	28	38	534	32	38	534	32	38	534
39	969	19	44	39	600	16	44	39	730	52	39	730	52	39	730
40	1050	28	64	40	621	—	80	40	766	42	40	766	42	40	766
				41	753	—	100	41	1278	50	41	1278	50	41	1278
				42	964	20	42	42			42			42	

¹ Arranged by WIA content.² The revised distillation method was used, *This Journal*, 25, 176 (1942).

TABLE 10.—Analysis of survey samples of butter¹ (1948)

CINCINNATI				ST. LOUIS				CHICAGO			
SAMPLE NO.	WIA	BUTTER ²	MOLD	SAMPLE NO.	WIA	BUTTER ²	MOLD	SAMPLE NO.	WIA	BUTTER ²	MOLD
1	mg/100 g fat 121	mg/100 g fat Trace	2	1	mg/100 g fat 163	mg/100 g fat Trace	6	1	mg/100 g fat 102	mg/100 g fat Trace	4
2	219	None	38	2	182	Trace	26	2	103	Trace	6
3	276	None	54	3	201	Trace	6	3	111	Trace	2
4	276	Trace	94	4	204		43	4	124	Trace	8
5	276	Trace	40	5	211		64	5	131	Trace	0
6	289	Trace	44	6	221	None	18	6	131	Trace	0
7	290	Trace	42	7	228	None	24	7	173	Trace	2
8	297	Trace	66	8	231	Trace	4	8	203	Trace	4
9	298	Trace	75	9	255	Trace	22	9	215	Trace	0
10	300	Trace	68	10	257	Trace	53	10	217	Trace	14
11	311	None	30	11	257	None	34	11	231	3.5	8
12	312	Trace	76	12	257	2.2	26	12	239	20.0	12
13	314	Trace	78	13	263	None	28	13	247	2.3	40
14	318	2.3	74	14	263		59	14	249	2.6	0
15	322	None	68	15	264	None	18	15	255	Trace	24
16	322	None	30	16	269	Trace	28	16	270	3.8	12
17	322	None	88	17	270	Trace	42	17	281	3.8	42
18	322	Trace	88	18	271	Trace	32	18	286	2.5	28
19	337	2.0	82	19	282		50	19	305	Trace	28
20	337	Trace	90	20	286	None	54	20	308	None	24
21	331	Trace	48	21	288		35	21	313	Trace	44
22	339	Trace	56	22	294		48	22	326	Trace	52
23	347	Trace	96	23	297	Trace	38	23	326	Trace	18
24	367	Trace	84	24	297	Trace	32	24	326	3.1	30
25	377	Trace	86	25	302	Trace	32	25	335	Trace	26
26	396	Trace	16	26	304	Trace	60	26	339	Trace	36
27	405	2.0	60	27	312	None	46	27	342	Trace	26
28	405	None	50	28	312		52	28	349	4.9	28
29	408	Trace	46	29	312	3.0	20	29	347	Trace	32
30	420	Trace	82	30	313	Trace	52	30	367	Trace	24
31	422	None	32	31	324	Trace	80	31	393	7.0	40

TABLE 10—(continued)

CINCINNATI				ST. LOUIS				CHICAGO			
SAMPLE NO.	WIA	BUTTER ¹	MOLD	SAMPLE NO.	WIA	BUTTER ¹	MOLD	SAMPLE NO.	WIA	BUTTER ¹	MOLD
32	mg/100 g fat	mg/100 g fat		32	mg/100 g fat	mg/100 g fat		32	mg/100 g fat	mg/100 g fat	
33	425	Trace	88	33	326	5.5	19	33	399	3.3	38
34	426	Trace	82	34	331	Trace	66	34	300	Trace	54
35	432	Trace	84	35	333	None	10	35	405	Trace	44
36	450	2.0	50	36	335	None	44	36	416	3.6	44
37	470	None	88	37	337	Trace	44	37	417	2.5	36
38	473	Trace	100	38	343	Trace	47	38	441	3.1	36
39	490	Trace	84	39	354	Trace	28	39	443	Trace	40
40	493	2.9	100	40	354	Trace	52	40	453	3.8	48
41	495	Trace	100	41	354	Trace	55	41	452	3.9	74
42	503	2.5	94	42	355	None	70	42	454	Trace	38
43	507	2.1	80	43	366	Trace	92	43	459	4.1	86
44	527	Trace	72	44	375	Trace	46	44	469	3.1	56
45	551	2.1	92	45	380	None	52	45	472	38.4	46
46	553	4.0	94	46	382	None	33	46	476	5.4	18
47	556	6.9	98	47	384	2.0	36	47	493	6.1	66
48	583	Trace	100	48	384	None	62	48	501	Trace	58
49	587	2.8	82	49	391	Trace	60	49	501	9.6	56
50	587	Trace	82	50	403	Trace	60	50	555	3.6	44
51	590	Trace	68	51	406	Trace	30	51	658	8.6	64
52	591	2.2	80	52	408	None	32	52	721	9.0	68
53	602	None	80	53	412	None	40	53	998	12.8	66
54	655	5.5	96	54	414	None	62	54	3299	7.9	78
55	663	5.5	100	55	418	Trace	47				
56	664	2.8	98	56	424	Trace	34				
	779	6.0	66	57	433	Trace	32				
				58	445	Trace	68				
				59	476	Trace	95				
				60	480	5.5	34				
				61	486	Trace	62				
				62	500	Trace	34				
				63	508	6.6	48				
				64	700		40				

¹ Arranged by WIA content.
² *This Journal*, 28, 944 (1946).

by the chemical analysis, which shows large quantities of WIA and also substantial amounts of butyric and propionic acids.

During the summer of 1947, a number of random samples of cream were obtained from centralizer-type creameries located in Cincinnati. These samples were said to represent the normal production and delivery made by an individual producer, and in each case the cream was unmixed with other producers' cream. The majority of the samples were delivered to the centralizer by means of route pick-ups. At the time this investigation was under way the usual time for route pick-up was every seven days. Thus, it is obvious that such seven-day cream, in most cases, contained cream from one, all the way up to seven, days old. Unless such cream had been properly refrigerated by the producer some decomposition was likely before delivery to the centralizer. The daily addition of newly separated cream to the can prior to the time of pick-up would naturally dilute the products of decomposition produced in older cream in the can. No information was available as to the sanitary conditions under which these creams were produced, nor as to whether the cream was hand skimmed or separated in a mechanical or water separator.

These creams were neutralized, pasteurized at 150°F for 30 minutes, cooled, and churned. The analytical data, together with comments, are given in Table 8. The results show that in a number of the creams decomposition actually did occur.

It is obvious that the 50 or so individual cans of cream comprising a commercial churn of butter will vary in their WIA content, depending upon the history, and therefore the condition, of the cream. Analysis of numerous cans of cream that were actually used in commercial churns has shown a variation in WIA of from less than 50 mg./100 g. of fat up to 6000 mg.

COMMERCIAL BUTTERS

As a part of this investigation two surveys of commercial butters were conducted. During the summer of 1945 a number of butters were purchased in the open market in Chicago, St. Louis, New Orleans, and Cincinnati, and examined for their WIA content. In each case mold count was also made, and in some cases butyric acid was determined. The data on the samples collected are given in Table 9.

In 1948, samples of butter were collected at creameries in the vicinity of Chicago, St. Louis, and Cincinnati, representing commercial grades of #1 and #2 butter. WIA, mold, and butyric acid were, in most cases, determined on each sample. Data obtained are given in Table 10.

The data in Tables 9 and 10 show that several of the samples contained large quantities of WIA, the amount in some cases even being very much in excess of that found in the authentic churns of butter churned from vat cream containing varying quantities of decomposed cream (1). Also substantial amounts of butyric acid were found in a number of the samples.

SUMMARY

These progressive decomposition experiments show that, as cream ages and decomposes, the fat may break down forming water-insoluble acids (WIA): in some cases in quantities far in excess of those normally present in sweet cream. Also butyric acid, and sometimes propionic acid, are often found in the samples of cream containing the larger quantities of WIA. It is suggested that the use of water separators might be one of the contributing factors causing early decomposition of cream. Data are presented on the determination of WIA in 321 samples of commercial butters. It has been shown that some of these samples contained materially larger quantities of WIA than were found even in authentic churns of butter known to have been churned from vats of cream which contained varying quantities of decomposed cream. Butyric acid was found in some of the butters containing the larger quantities of WIA.

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RAPID DETERMINATION OF SULFUR DIOXIDE IN WINE*

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A review of the literature indicates extensive work on methods for determining the sulfur dioxide content in beverages and foods. Reports by L. V. Taylor on sulfur dioxide determinations in wine and beer (1, 2, 3), reviews by Nichols and Reed (4) and Monier-Williams (5) evaluate some of the methods in use. Volumetric, gravimetric, and titrimetric methods have been generally employed in determining sulfur dioxide in wine. No attempt is made in this paper to compare the relative merits of the above-mentioned methods nor to draw a comparison between them and the method outlined below.

The experimental work on wine samples reported herewith indicates some rather interesting facts, but is not based on a sufficient number of analyses and does not cover a range of conditions suitable for drawing

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 10-12, 1949.

definite conclusions. Possible oxidations and reductions during distillation were considered, but the scope of such side reactions is not discussed. Mention only is made of the fact that addition of oxalic acid or sodium arsenite to the solutions before distillation seems to make for higher and more consistent sulfur dioxide recoveries.

In wines, sulfur dioxide, when used as a preservative, occurs both in the free and combined forms. In bottled beverages the content of sulfur dioxide is not constant but decreases with time, even when the containers are tightly sealed (6, 7) In the chemical examination of wines both the total and the free sulfur dioxide concentration is desired.

•Upon distillation, the free sulfur dioxide passes into the distillate and is only partially absorbed, unless a suitable absorbing medium is used. The absorption medium used in the following procedures is a dilute solution of lead acetate. The salt, lead sulphite, which is formed by interaction of sulfur dioxide and lead acetate, is highly insoluble and forms a colloidal

TABLE 1.—*Spectral transmittance through lead sulphite suspension**

WAVE LENGTH $m\mu$	TRANSMITTANCE
	<i>per cent</i>
400	32.0
450	36.8
500	41.7
550	46.6
600	51.3
650	56.2
700	61.0

* Sulfur dioxide content about 25 mg./liter.

suspension. At concentrations of 100 mg./liter or less (calculated as sulfur dioxide) the stability of the colloid is sufficient to permit photometric measurements. The optical density of the suspension is also a limiting factor at about this same concentration. After photometric measurements have been made, the same distillate may be used in iodimetric titrations. This test is hereafter referred to as the "Lead Sulphite Method" to distinguish it from other methods mentioned.

A Coleman D. M. Spectrophotometer, Model 10-S, was used to determine a suitable wave length for making photometric measurements on colloidal suspensions of lead sulphite. Table 1 gives the per cent spectral transmission at various wave lengths from 400–700 $m\mu$. Figure 1 shows wave length plotted against per cent transmission. The resulting straight line indicates that the selection of the wave length of light for the transmission measurements is not critical.

From the standpoint of the volatile acids, exclusive of sulfur dioxide, in wine distillates it is immaterial if a portion of sulfur dioxide escapes. The amount absorbed in the distillate gives the correction to be applied to volatile acid figure.

Free sulfur dioxide is readily given off on distillation so that portions of the distillate used in alcohol determination may be utilized in testing for sulfur dioxide. The "alcohol distillate" is selected instead of the "volatile acid distillate" because it has the same volume as that of the wine sample, whereas, upon quantitatively steam distilling the volatile acids, the quantity of the distillate is often several times as great as the original sample. The concentration of acetic acid and ethyl alcohol in wine distillates affects the solubility of lead sulphite, but the error therefrom does not prohibit the use of the "alcohol distillate" in approximating the free sulfur dioxide content.

A series of tests utilizing several fractions of wine distillates show that

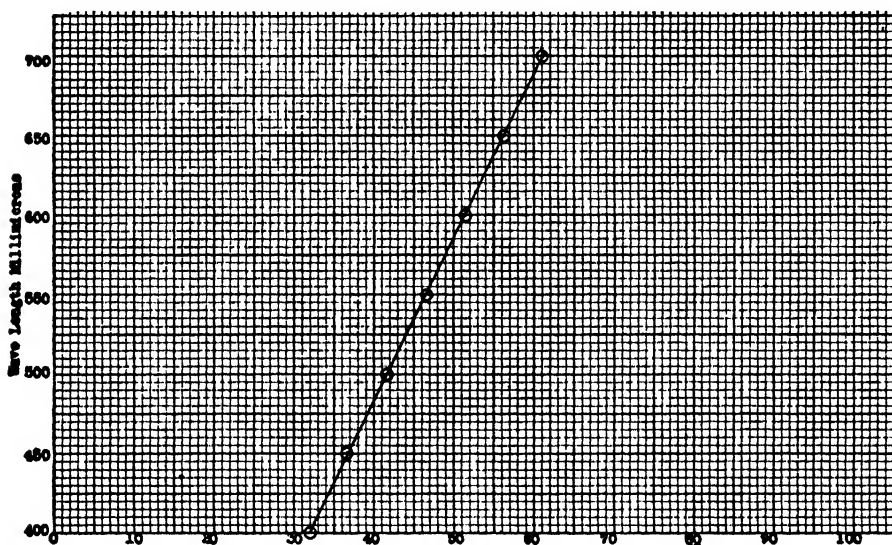


FIG. 1.—Per cent spectral transmittance through lead sulphite suspension.
Negative log transmittance Coleman spectrophotometer

free sulfur dioxide is removed early in the distillation. An approximation of the quantity of free sulfur dioxide in distillates may be accomplished very rapidly by the procedure outlined below.

METHOD

To 10 ml of distillate from the alcohol determination add 0.5 ml of 5% neutral lead acetate soln. Compare the cloud produced with that of standards containing from 5–100 mg/liter of sulfur dioxide. If the distillate contains quantities greater than 50 mg/liter it should be diluted with distilled water until it approaches this value. The optical density may be obtained by use of a colorimeter or photometer. For photometric use a standard curve may be prepared and used henceforth.

The value obtained cannot be considered the free sulfur dioxide content of the wine, but it is of comparable magnitude to that in the volatile acid distillate and may be applied as a correction in expressing the volatile acids, exclusive of sulfur dioxide.

The above method provides a means of rapidly screening a number of samples to select those which do contain appreciable amounts of sulfur dioxide.

Table 2 shows the sulfur dioxide values obtained, with alcohol distillates by comparison method, photometrically and iodimetrically; and iodimetrically with the volatile acid distillate.

TABLE 2.—*Sulfur dioxide in distillates of commercial wines*

WINE SAMPLES	ALCOHOL DISTILLATE			ACID DISTILLATE, IODIMETRIC
	COMPARISON	PHOTOMETRIC	IODIMETRIC	
Muscadine	0 mg/l	0 mg/l	0 mg/l	0 mg/l
Muscadine	30	30	32	30
Catawba	60	55	58	62
White Bordeaux	120	138	132	116
(imported)				
Red Bordeaux	35	30	32	38
(Imported)				
Red Burgundy	5	3	4	4
Blackberry	130	116	115	120

Distillates containing more than 60 mg/l of SO_2 were diluted 50% with water for comparison and photometric tests.

PREPARATION OF COMPARISON STANDARDS AND A STANDARD CURVE

Sulphite solutions are not stable over extended periods of time and must be prepared immediately before use. About 200 mg. of sodium acid sulphite is added to 100 ml. of water acidified with sulfuric acid (3 ml.), and sulfur dioxide is distilled through a reflux condenser into a series of five receivers containing 50 ml. of 1% lead acetate solution. The receivers were changed so that gradations from a faint cloud to a milky suspension were obtained. The volume in each receiver was brought up to 100 ml. with distilled water.

The suspensions were examined with both the Coleman spectrophotometer at wave length 600 $\text{m}\mu$ and the neutral wedge photometer employing a green filter No. 56. After completion of the optical examination the sulfur dioxide was determined by acidifying with 5 ml. of concentrated hydrochloric acid and titrating with 0.02 *N* iodine solution using starch indicator. Table 3 shows this data. In Figure 2 the concentration of sulfur dioxide is plotted against the negative log of the transmission. In Figure 3 the neutral wedge photometer readings are plotted against the concentration of sulfur dioxide.

Photometric and turbidimetric standards may be prepared from aqueous suspensions of lead phosphate. Either phosphoric acid or monosodium phosphate appear satisfactory. Stock solutions of either are fairly stable and will not have to be freshly prepared each time they are used.

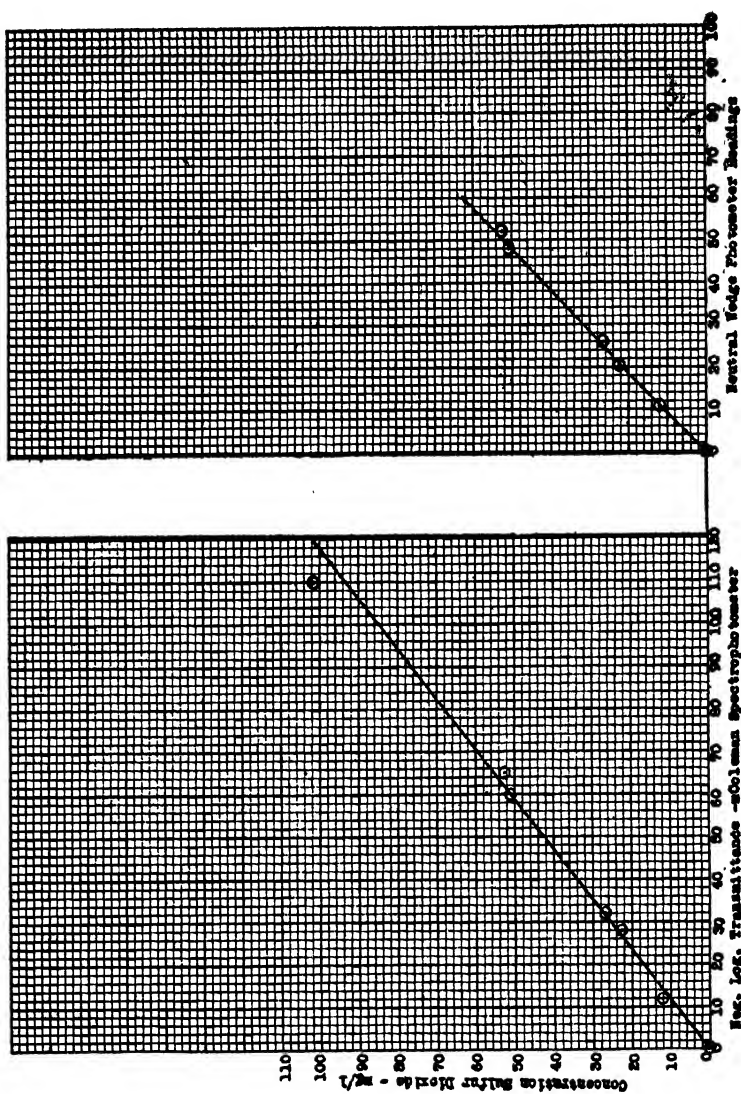


Fig. 2.—Standard curve for SO₂ determination

Fig. 3.—Standard curve for SO₂ determination

TABLE 3.—*Photometric standards for sulfur dioxide*

SAMPLE NO.	NEUTRAL WEDGE PHOTOMETER	COLEMAN SPECTRO- PHOTOMETER TRANSMISSION	NEG. LOG. TRANSMISSION	IODIMETRIC SO ₂ mg/l
0	0	100.0%	0	0
1	11.0	75.7	0.12	12.0
2	20.5	52.5	0.28	22.5
3	26.0	47.8	0.32	26.5
4	52.0	22.0	0.65	52.5
5	not readable	7.9	1.10	101.5
5*	48.0	25.1	0.60	(50.8)

* Diluted sample No. 5 one-half with distilled water.

Neutral lead acetate, 5% solution, is added directly to the phosphoric acid or sodium phosphate solution. Turbidity is compared with that of lead sulphite suspensions.

DETERMINATION: TOTAL SULFUR DIOXIDE IN WINE

To 50 ml. of wine sample are added boiling stones and 50 ml. of 5% sulfuric acid. The receiver consists of a graduate containing 50 ml. of 1% neutral lead acetate solution. The end of the condenser or adapter is immersed about two inches below the surface of the liquid in the receiver. About 50 ml. of distillate is collected and the volume made up to 100 ml. with distilled water. After optical examination the suspension is acidified with hydrochloric acid and titrated with 0.02 *N* iodine. A small amount of lead sulphite usually adheres to the adapter or condenser and must be washed into the titration flask with acid. Table 4 shows the results obtained on a number of wines by the "Lead Sulphite Method" using the Coleman Spectrophotometer followed by iodimetric titration and a check employing Monier-Williams method (8), both the titrimetric and gravimetric results being reported.

Distillation of wines in the preceding manner renders the photometric

TABLE 4.—*Total sulfur dioxide in wine distillates*

WINE SAMPLES	MONIER-WILLIAMS		LEAD SULPHITE PHOTOMETRIC	METHOD IODIMETRIC
	TITRIMETRIC	GRAVIMETRIC		
Blackberry	0.0 mg./l.	0.0 mg./l.	0.0 mg./l.	0.0 mg./l.
Blackberry (603.8 mg./l. SO ₂)	608.0	600.6	—	602.8
Grape Catawba	12.8	18.0	13.0	17.9
Zinfandel	30.4	32.0	26.0	30.8
Burgundy (White)	132.0	138.0	120.0	140.7
Sauterne	60.4	68.4	60.0	67.6

test for sulfur dioxide considerably less accurate than iodimetric titrations. Tests on a number of wines which had undergone additional acetic fermentation gave sulfur dioxide values as low as one-half that obtained by iodimetric titration or the Monier-Williams determination. This may have been due to the excess acetic acid in the distillate partially solubilizing the lead sulphite. Those same wines when distilled under reflux after addition of oxalic acid or sodium arsenite, gave photometric values closely approximating those by iodimetric titration and the Monier-Williams method. The use of a current of air through the distillation apparatus to aid in driving over sulfur dioxide caused no appreciable variation in the recovery of sulfur dioxide, so it is suggested that a reducing agent added to wine sample may prevent oxidation of the sulfur dioxide by air during distillation.

SUMMARY

A photometric method of determining sulfur dioxide in wine distillate is presented.

Neutral lead acetate solution is shown to be a good absorption medium for sulfur dioxide.

Lead sulphite suspensions may be acidified and the sulfur dioxide content determined by iodimetric titration.

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AN ULTRAVIOLET SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF BENZENE HEXACHLORIDE*

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Immediately following the introduction of benzene hexachloride as an insecticide, there arose a host of analytical problems related to the determination of this compound and its individual isomers.

* Part of the material in this paper was presented before the American Society of Pharmacology and Experimental Therapeutics, Federation Meetings, Detroit, 1949. The data are taken from the dissertation submitted by Bernard Davidow to the Faculty of the Graduate School, Georgetown University, 1948, in partial fulfillment of the requirements for the degree of Master of Science.

Although analytical methods based on infrared spectrophotometry (1) partition chromatography (2) and on total or hydrolyzable chlorine (3) were available, they were not considered applicable to the determination of benzene hexachloride in biological tissues or spray residues since they lacked either sensitivity or specificity. Therefore, a method more suitable for the specific estimation of the relatively small quantities of benzene hexachloride which might occur in biological tissues or spray residues was sought.

Advantage was taken of the following observations in developing the proposed method. Mitscherlich (4) in 1833 described the formation of trichlorobenzenes from benzene hexachloride by treatment with alkali. Van der Linden (5) studied the products of alkaline hydrolyses of the alpha, beta, and gamma isomers of benzene hexachloride and by a method of mixed melting points estimated that of the mixture of 1, 2, 3-, 1, 3, 5-, and 1, 2, 4-trichlorobenzene formed, approximately 82 per cent was the 1, 2, 4-trichlorobenzene. Furthermore, Conrad-Billroth (6) in 1932 reported that the 1, 2, 4-trichlorobenzene had an absorption spectrum in the ultraviolet region characteristically different from the other trichlorobenzenes.

The method as finally evolved consists of the initial extraction of the insecticide from the material to be examined, the subsequent conversion of the benzene hexachloride with alkali to 1, 2, 4-trichlorobenzene, the purification of this latter compound and its estimation by measurement of its optical density at 286 millimicrons.

METHOD

APPARATUS

- (1) Beckman quartz spectrophotometer with ultraviolet attachments and quartz cells with 1 cm. absorption path.
- (2) Refluxing apparatus: 125 ml. flasks, condensers (all connections standard taper joints).
- (3) Soxhlet apparatus.
- (4) Chromatographic tubes, 20 cm. long, 2 cm. O.D.: 30 cm. long, 5 cm. O.D.

REAGENTS

- (1) *Benzene hexachloride, gamma isomer.*¹
- (2) *Ether*, ACS.
- (3) *Sodium sulfate anhydrous powder.*
- (4) *Methanolic potassium hydroxide*, 1.5 N.—Dissolve 98 g of potassium hydroxide C.P. in absolute methanol C.P. to make one liter of solution.
- (5) *Magnesium oxide activated*. No. 2641, Westvaco Chlorine Products Corp.
- (6) *Aluminum oxide anhydrous*.—Merck.
- (7) *Celite* No. 545.²
- (8) *Silica gel activated*.—Mesh size 28–200. Davison Chemical Corporation. Reactivate after use by washing with alcohol, followed by water and then

¹ Lindane, manufactured by Hooker Electrochemical Company.

² Manufactured by Johns Manville Company.

heating in oven at 400° for 24 hours. (*Caution:* All organic solvents must be removed before putting in oven.)

- (9) *Normal hexane, commercial.**—Purify by passage through silica gel column (500 g silica gel will purify 500 ml hexane). (7) In case the unknown is passed thru an alumina or magnesium oxide column the hexane used for the blank should be similarly purified.

PREPARATION OF STANDARDS

- (1) Prepare a standard soln of benzene hexachloride, gamma isomer in absolute methanol to contain 1 mg per ml. To five 125-ml flasks with ground glass condensers add first 0, 1.00, 2.00, 4.00, and 8.00 ml, respectively, of the standard soln.
- (2) Reflux with 20 ml of 1.5 *N* methanolic potassium hydroxide for one hour on a steam bath.
- (3) Cool, then transfer to a separatory funnel with 25 ml normal hexane and 250 ml of distilled water.
- (4) Shake for 2 min., allow the two phases to separate, and discard the aqueous phase.
- (5) Wash hexane phase with ten 400-ml portions of distilled water without shaking, by pouring each portion in a slow stream into the soln.
- (6) Dry the hexane soln by filtering thru 10–12 g of anhydrous sodium sulfate which has been previously wet with hexane and adjust volume to 25 ml (a 40-mm. chemical funnel is satisfactory for filtration).
- (7) Determine optical density of the standards at wave lengths 284, 286, and 290 millimicrons.

DETERMINATION

- (1) Extract sample containing between 0.5 and 15 mg of benzene hexachloride with ether (for 10 p.p.m. a 200-g sample is suitable).
 - a) *Biological tissue*—Place weighed tissue in a mortar, add sodium sulfate equal to about three times the weight of the tissue. Grind to a coarse dry powder to insure dehydration and complete breaking up of the cells. Transfer to a Soxhlet and carry out extraction for a period corresponding to at least 10 syphonings of the apparatus.
 - b) *Dry materials*, such as animal laboratory feed, are ground and extracted directly without dehydration.
- (2) Transfer ether soln to flask to be used for hydrolyses and evaporate the ether, using a current of air.
- (3) Reflux the ether extract with 20 ml of 1.5 *N* methanolic potassium hydroxide for one hour on a steam bath.
- (4) Cool, then transfer to a separatory funnel with 25 ml normal hexane and 250 ml of distilled water.
- (5) Shake for 2 min., allow the two phases to separate, and discard the aqueous phase.
- (6) Wash hexane phase with ten 400-ml portions of distilled water without shaking, by pouring each portion in a slow stream into the soln.
- (6) Dry the hexane soln by filtering thru 10–12 g of anhydrous sodium sulfate which has been previously wet with hexane and adjust volume to 25 ml (a 40-mm chemical funnel is satisfactory for filtration).
- (7) (a) *Tissues (except liver)* from rats and dogs require no further purification.

* Phillips Petroleum Company.

(b) *Liver*—Purify extracts from liver by passage thru a magnesium oxide column:

Pass the hexane soln from 5 g liver thru a column 3–4 cm long and 2 cm in diameter, containing equal parts of magnesium oxide and celite (packed dry), and wet with hexane before adding the extract. (To decrease the chance of disturbing the surface of the column about 4 g of anhydrous sodium sulfate may be put on top before adding the solvent.)

(c) *Diet*—For commercial laboratory diet pass the hexane soln thru a column of alumina (same size, and packed as in (b)).

Collect 5 ml fractions of eluate in 10 ml glass-stoppered graduates. Transfer the fourth 5-ml portion to the absorption cell.

- (8) Determine optical density of the unknown at wave lengths 284, 286, and 290 millimicrons using the hexane (reagent 9) as a blank. Compare with a standard curve prepared from gamma benzene hexachloride. Calculate the concentration of benzene hexachloride in the unknown.

OBSERVATIONS

It was noted that hexane solutions of the nonsaponifiable fractions of laboratory diets, spinach, potatoes, apple wax, and biological tissues, free from benzene hexachloride, had absorption properties in the ultraviolet region which interfered with a quantitative determination of benzene hexachloride unless treated as outlined above. To overcome this difficulty, it was necessary to characterize the spectrum of the components which contribute to the expression of density as the critical wave length (286 millimicrons). Therefore spectral absorption curves were prepared on the nonsaponifiable fraction of these materials and on the products of alkaline hydrolysis of the isomers of benzene hexachloride. (Figures 1 and 2).

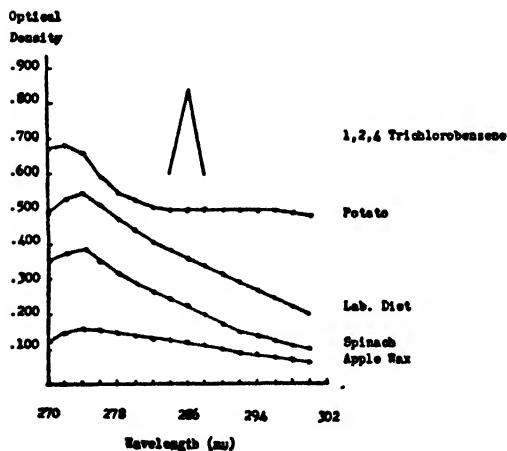


FIG. 1.—Background spectra of Nonsaponifiable fractions.

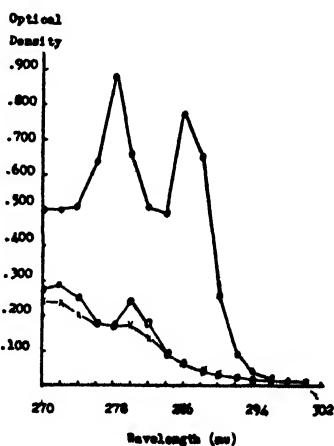


FIG. 2.—Absorption curves of the trichlorobenzenes in hexane
 —1,2,4—Trichlorobenzene 250 $\mu\text{g/ml}$
 ○—1,3,5—Trichlorobenzene 250 $\mu\text{g/ml}$
 ×—1,2,3—Trichlorobenzene 250 $\mu\text{g/ml}$

These data reveal that the spectral absorption curves of the control material are linear through the range of 284 to 290 millimicrons. On account of this linearity, resolution of the components contributing to the density at 286 millimicrons may be accomplished by two methods. The first consists of noting the optical densities at wave lengths 284 and 286 millimicrons and applying simultaneous equations for the resolution of a two component color system.

Derivation and Application of a Formula for the Resolution of a Two Component Color System

Legend:

a = ratio of optical density at wave lengths 284/286 due to benzene hexachloride.

b = ratio of optical density at wave lengths 284/286 due to background.

X = optical density due to benzene hexachloride.

Y = optical density due to background.

D = optical density.

From Beers Law.

$$(1) D_{284} = X_{284} + Y_{284}$$

$$(2) D_{286} = X_{286} + Y_{286}$$

Using ratios a and b and substituting in equation (1)

$$(3) D_{284} = aX_{286} + bY_{286}$$

Multiply equation (2) by b

$$(4) bD_{286} = bX_{286} + bY_{286}$$

Subtract equation (4) from (3)

$$(5) D_{284} - bD_{286} = aX_{286} - bX_{286}$$

$$(6) D_{284} - bD_{286} = X_{286}(a - b)$$

$$(7) X_{286} = \frac{D_{284} - bD_{286}}{a - b}$$

A second method which can be applied for the elimination of spectral absorption due to other substances is the base-line method (8). In this case, optical density readings at wave lengths 284, 286, and 290 millimicrons are plotted graphically. A line called the base line is ruled between the points at wave length 284 and 290 millimicrons. The distance between the point at 286 millimicrons for an unknown or standard and the point on the line directly below it is called the base-line density. The base-line density (Fig. 3) is directly proportional to the concentration of benzene hexachloride. This method of analysis can be adapted with slight modification to the determination of benzene hexachloride in many substances. To do so, a spectral absorption curve must be plotted on control samples free of benzene hexachloride, so as to be certain that the background absorption spectrum is linear through the critical wave lengths. Stopcock lubricants may contribute to the optical density in the ultraviolet region, and therefore should be avoided.

With some substances the determination of benzene hexachloride may be complicated by the presence of materials which have a great deal of

absorption in the ultraviolet region and may raise the optical density readings above the optimum range, or may affect the linearity of the spectral absorption curve through the critical wave lengths. Many of these substances, however, can be removed by chromatographing with the proper adsorbent. For example, in the determination of benzene hexachloride in liver tissue or butter, interference was encountered by the presence of vitamin A. The interference has been eliminated in this laboratory by chromatographing the hexane solution containing the vitamin A and the 1, 2, 4-trichlorobenzene through magnesium oxide(9). The vitamin A is strongly held on the adsorbent while the 1, 2, 4-trichlorobenzene is recovered in the hexane eluate. In a similar manner, many plant pigments may be adsorbed on a chromatographic column of alumina.

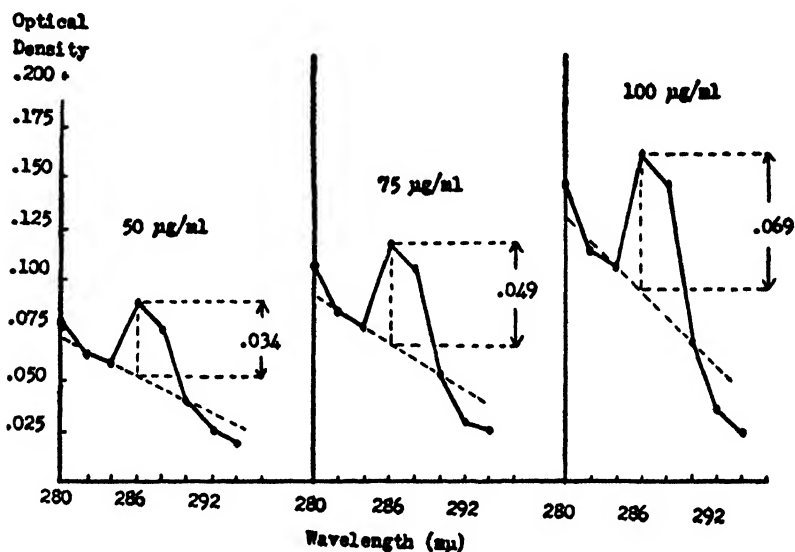


FIG. 3.—Estimation of benzene hexachloride by base-line technique.

The recoveries of benzene hexachloride added to animal laboratory diet and rat fat are illustrated in Tables 1 and 2. The hexane containing the trichlorobenzenes and plant pigments from the laboratory diet was chromatographed through a column of alumina. In the case of rat fat, the hexane solution needed no such purification.

An analysis of the recovery data reveals a standard deviation of 8%. The source of error may be due in part to:

- (1) Loss of benzene hexachloride and 1, 2, 4-trichlorobenzene, because of their volatility.
- (2) Emulsification of a portion of the 1, 2, 4-trichlorobenzene and its discard with the aqueous wash.

The method as described is of an empirical nature; therefore, the analy-

sis should be performed under the same conditions as used for the preparation of the standard curve. In this manner, as little as 500 micrograms of benzene hexachloride per total sample may be determined in biological tissues, in spray residues on vegetables such as spinach and cabbage, and in animal laboratory diets.

TABLE 1.—*Recovery of benzene hexachloride added to animal laboratory diet*

ISOMER	ADDED	RECOVERED	PER CENT RECOVERED
	p.p.m.	p.p.m.	
Alpha	100	100	100
Alpha	100	100	100
Beta	100	106	106
Beta	100	112	112
Gamma	100	92	92
Gamma	100	101	101
Delta	100	101	101
Delta	100	101	101

TABLE 2.—*Recovery of benzene hexachloride added to 1 gram of rat fat*

ISOMER	ADDED MICROGRAMS	RECOVERED MICROGRAMS	PER CENT RECOVERED
Alpha	500	500	100
	1000	1120	112
	2000	1720	86
Beta	1000	1010	101
	1500	1243	83
	2000	1760	88
Gamma	1000	900	90
	1500	1400	93
	2000	1750	88
Delta	1000	1000	100
	1500	1460	97
	2000	2040	102

SUMMARY

A method for the determination of benzene hexachloride has been presented. The method is based upon the conversion of the benzene hexachloride to 1, 2, 4-trichlorobenzene and the estimation of the trichlorobenzene with an ultraviolet spectrophotometer.

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AN ULTRAVIOLET SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF BENZENE HEXACHLORIDE IN MILK

By JOHN P. FRAWLEY and BERNARD DAVIDOW (Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D.C.)

An ultraviolet spectrophotometric procedure for the quantitative estimation of benzene hexachloride has been developed by Davidow and Woodard (1). The method of these workers is based on the dehydrohalogenation of benzene hexachloride to 1,2,4-trichlorobenzene and the estimation of the latter compound by means of an ultraviolet spectrophotometer. The method is applicable to all the isomers.

Recently, when applying the method to milk analyses, several difficulties were encountered which led to (1) a unique procedure for the extraction of fat from milk samples and (2) modifications of the original method to yield greater sensitivity, 0.5 p.p.m. on a 200 ml. sample of milk.

Since the method of Davidow and Woodard (1) is based on the conversion by alkali of the benzene hexachloride to trichlorobenzene, the preferred method of analysis would be one capable of distinguishing the benzene hexachloride from any possible trichlorobenzene present in milk, assuming that some dehydrohalogenation of the insecticide may take place in the animal. In order to detect the presence of 1,2,4-trichlorobenzene in milk, an ultraviolet spectrophotometric examination of a steam distillate of the extracted fat may be used. Thus, any treatment with alkali before or during the extraction, with the possible conversion of benzene hexachloride to trichlorobenzene, was undesirable. If preliminary analysis showed the absence of trichlorobenzene, then any benzene hexachloride can be determined by alkaline conversion to trichlorobenzene, as described below. If, however, the preliminary analysis indicated the presence of trichlorobenzene, the benzene hexachloride content could

be determined by the increase in trichlorobenzene after treatment with alkali.

Since benzene hexachloride is stable in the presence of acid, various methods of treatment with acids were tried in an attempt to minimize the tendency for the milk to emulsify when shaken with an organic solvent. In all cases the protein material in the milk caused sufficient emulsification to require centrifugation in order to separate the solvent layer from the milk. For milk samples of about 200 ml., it was considered preferable to use the procedure described below rather than centrifuge large volumes.

The procedure finally employed consisted in adjusting the *pH* of the milk to approximately 4.6, the isoelectric point of casein. This enabled the precipitated protein and the fat, which was quantitatively occluded on the protein, to be separated from the liquid portion of the milk. Extraction of the fat from the precipitated protein was then carried out in a Soxhlet apparatus. This method of treatment and extraction was found to be equally effective for both regular milk and homogenized milk. It also eliminated centrifugation and offered no strict limitation to the sample size.

Glacial acetic acid was employed and 0.75 ml. per 100 ml. of milk was found to give a *pH* of 4.5. Most rapid precipitation and flocculation were obtained when the milk was first diluted with an equal volume of distilled water and stirred slowly during the addition of the acetic acid.

The precipitate was then collected on a Büchner funnel. The filtrate frequently foamed during filtration, but this was controlled by the addition of an antifoaming agent, *i.e.*, isoamyl alcohol. Extraction of the aqueous filtrate with ether yielded only 0.01 gram of residue per 100 ml. of milk and therefore was not considered necessary for quantitative recoveries.

The casein-fat residue was ground and dried with anhydrous sodium sulfate and extracted with ether in a Soxhlet apparatus. As a general rule 20 grams of sodium sulfate per 100 ml. of milk yield a coarse dry power. However, if the residue has not been adequately dried on the Büchner funnel, a considerably larger quantity of the drying agent is required.

Fat recoveries determined by this precipitation and extraction procedure were compared with the A.O.A.C. Method of Roese and Gottlieb and the results are presented in Table 1. The average recovery was found to be 97 per cent.

After extraction in a Soxhlet apparatus with ether, the ether is evaporated and the fat saponified with 25 ml. of 1.5 *N* methanolic potassium hydroxide per 100 ml. of milk. This quantity is in excess of the amount required for the saponification of average milk samples (3.5 to 4.5% butter fat). By titration of the excess potassium hydroxide, it was found that 25 ml. of 1.5 *N* methanolic potassium hydroxide will saponify approximately

7 grams of butter fat. Therefore, this quantity of alkali is sufficient except in extreme cases where the milk contains more than 7% butter fat.

During saponification, dehydrohalogenation of the benzene hexachloride is affected, resulting chiefly in the formation of 1,2,4-trichlorobenzene (2, 3). The saponified material is then shaken with normal hexane to extract the trichlorobenzene. After washing and chromatographing the hexane solution, it is concentrated to 5 ml. with gentle boiling (caution: without a stream of air.)

The optical density of the concentrated hexane solution is determined at 284, 286, and 290 millimicrons and the quantity of benzene hexachloride

TABLE 1.—*Fat recoveries as compared with A.O.A.C. method*

EXPERIMENTAL METHOD	A.O.A.C. METHOD	RECOVERY
<i>g. fat/100 ml milk</i>	<i>g. fat/100 ml milk</i>	<i>per cent</i>
3.74*	3.89*	96
5.23	5.52	95
4.17	3.97	105
3.64	4.00	91
3.60*	3.70*	97

* Indicates homogenized milk sample.

is determined by a base-line technique (4). The selection of the wave lengths 284, 286, and 290 millimicrons was made because the base line obtained through this range for control milk samples was found to yield a straight line. Readings made at other wave lengths on the same blank sample indicated a variance from a straight line and would consequently result in slight positive base-line densities on blank samples.

The base-line technique consists in graphically plotting wave length against optical density. The optical density readings at 284, 286, and 290 millimicrons are located on the graph. A straight line, called the base line, is constructed through the points at wave lengths 284 and 290. The distance from the point at 286 millimicrons and the point on the line directly below it, is called the base-line density. The quantity of benzene hexachloride in the unknown can be estimated from a standard curve relating base-line density to concentration of benzene hexachloride.

A mathematical resolution for the determination of the base-line density may also be employed. Using the wave lengths previously indicated, the following formula may be adopted:

A = optical density at 284 millimicrons

B = optical density at 286 millimicrons

C = optical density at 290 millimicrons

$$\text{Base-line density} = B - \left(A - \frac{A - C}{3} \right)$$

Comparison of the base-line density with the standard curve establishes the concentration of benzene hexachloride.

METHOD

Apparatus and reagents are the same as those used in the preceding paper with the addition of a special* Erlenmeyer flask with a 10 ml. tube, graduated at 5 ml., sealed to the side of the flask near the bottom.

PROCEDURE (FOR 200 ML. OF MILK)

- (1) Dilute 200 ml of milk with 200 ml of water.
- (2) Add 1.5 ml of glacial acetic acid, stirring slowly—allow precipitate to settle for twenty minutes.
- (3) Filter the precipitate through an approximately 1 or 2 mm layer of celite on a Büchner funnel (18 cm size is convenient).
- (4) Grind precipitate to a coarse dry powder with anhydrous sodium sulfate.
- (5) Extract dry powder with ether for 2 hours in a Soxhlet apparatus (a minimum of 20 syphonings of the apparatus).
- (6) Transfer ether extract of 125 ml flat-bottom flask (standard taper) and evaporate the ether on a steam bath using a gentle stream of air.
- (7) Add 50 ml of 1.5 *N* methanolic potassium hydroxide and reflux for 1 hour.
- (8) Cool and transfer quantitatively to a 500 ml separatory funnel with 25 ml of normal hexane and 250 ml of distilled water. Shake for two minutes and allow the two phases to separate.
- (9) Discard the aqueous phase and wash the hexane phase without shaking with ten 400 ml portions of distilled water.
- (10) Dry the hexane by filtering through anhydrous sodium sulfate which has been previously wet with hexane. Wash the sodium sulfate with three 5 ml portions of hexane.
- (11) Pass the hexane solution through a column containing a 1-1 mixture of magnesium oxide and celite, followed by a 15 ml wash with hexane.
- (12) Transfer the eluate to the Erlenmeyer flask with the graduated side arm and concentrate the hexane solution to 5 ml. by gentle heating on a steam bath using a 10 cm long air condenser. This volume can be easily determined by running the solution into the graduated side arm. In case this special flask is not available, transfer the evaporated solution to a 10 ml volumetric flask and make to volume. This will decrease the sensitivity of the procedure by one-half.
- (13) Determine the optical density of the hexane solution at 284, 286, and 290 millimicrons.
- (14) A line called the base line is ruled between the points at wave lengths 284 and 290 millimicrons. The distance between the point at 286 millimicrons and the point on the line directly below it is called the base-line density.
- (15) The quantity of benzene hexachloride in the unknown is estimated from a standard curve relating base-line density with concentration of benzene hexachloride.

The performance of the method is illustrated by the following recovery experiments using regular milk and homogenized milk (Table 2). An ether solution of the benzene hexachloride was added to milk and the ether evaporated with a stream of air. The average recovery was found to be 96.1 per cent.

* Use of the flask was suggested by Mr. L. Tufts of Hooker Electrochemical Co., Niagara Falls, N. Y

TABLE 2.—*Recoveries of benzene hexachloride added to control milk*

MG. ADDED TO 200 ML. MILK	MG. RECOVERED		PER CENT RECOVERED	
	REGULAR	HOMOGENIZED	REGULAR	HOMOGENIZED
1.00	0.99	0.97	99	97
0.60	0.58	0.58	97	97
0.40	0.37	0.37	93	93
0.20	0.18	0.19	90	95
0.10	0.10	0.10	100	100

SUMMARY

A method for the determination of benzene hexachloride in milk has been presented. The method is sensitive to 0.1 mg. of benzene hexachloride and, as outlined, is capable of quantitatively estimating 0.5 p.p.m. in milk.

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THE RELATION BETWEEN FILTH IN WHOLE FIGS
AND FILTH RECOVERED FROM FIG PASTE*

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A method of sampling, examination and classification of the filth in bulk figs has been described by Howard (1). Hodges (2) extended the work of Howard and introduced a gasoline flotation technique for the estimation of filth in bulk figs. He showed that a much better recovery of insects, particularly of whole larvae, was obtained by the gasoline flotation technique than by the macroscopic method described by Howard. This was confirmed by work in progress in this laboratory designed to adopt the flotation method to a quantitative procedure for bulk figs. Hodges (2) showed that the gasoline flotation was also applicable to the estimation of filth in fig paste but thus far it has not been placed on a quantitative basis.

The work presented here was undertaken to determine the relationship between the estimation of filth in bulk figs by the macroscopic method

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and the estimation of filth in fig paste by the gasoline flotation method. The determination of this relationship appeared of interest for the evaluation of the filth content of fig paste in terms of the filth content of bulk figs from which it was produced.

MATERIALS AND METHODS

Six bags or boxes each containing 25 to 30 pounds of Smyrna or Calimyrna figs (1948 crop) were obtained from each of five different lots of bulk figs at the time of importation. A random sample of 200 figs was drawn from each bag of each lot and examined for filth by the method described by Howard (1) using a dissecting microscope (Greenough type). Each type of defect was recorded for each individual fig examined, and after examination the fig was returned with its defect to the bag from which it was drawn. In lot 4 the identity of the original bags was not kept, and the examination was made on a sample of 200 figs drawn from the pooled lot. After examining the five lots of bulk figs they were sent to a firm engaged in processing such products, and fig paste was prepared from them. Each lot was processed separately and three boxes of paste of approximately 60 pounds each were obtained from each.

Samples of six cores or plugs of 40 to 60 g. each were taken by means of a butter trier from each of the boxes of each lot. The individual plugs were placed in separate beakers with about 1000 ml. of water, and the mixture boiled for approximately 30 minutes until the paste was thoroughly dispersed. The mixture was then transferred to a 4000 ml. beaker and about 30 ml. of white, lead-free gasoline was added. This was stirred vigorously and the beaker was filled with cold water and allowed to stand until the gasoline layer rose to the top. The gasoline layer and approximately 2000 ml. of the water were transferred to a Wildman trap flask and the gasoline layer trapped off and filtered by means of a Büchner funnel. The flotation was repeated on the mixture, and the gasoline layer filtered through the same paper. The combined residues from each core were then examined for insects, insect heads and larvae, using a Greenough type microscope at a magnification of $7\times$ to $15\times$. The number of heads and whole insects or larvae found was expressed as heads per 100 g. of fig paste. Insect fragments, such as legs and wings, were recorded but were disregarded in the results reported here. A second series of six-core samples was taken and examined in like manner, except that 100 g. cores were drawn in order to determine if the above extrapolation procedure was justified.

RESULTS

Table 1 shows the results obtained in the examination of each box of each lot of bulk figs. It is noted that of the five lots the mean per cent defective varied from 25.2 per cent to 5.4 per cent. The number of defectives in the 200 figs examined from each box in each lot showed con-

siderable variations; however, an analysis of variance showed no significant difference between boxes within each lot. The standard deviation for lot 4 is higher than the others because of the smaller number of figs examined in the lot.

TABLE 1.—*Filth content of each box of bulk figs used for paste*

BOX NO.	NO. FIGS. EXAMINED PER BOX	NO. DEFECTIVES FOUND IN				
		LOT 1	LOT 2	LOT 3	LOT 4	LOT 5
1	200	59	48	46	35	13
2	200	61	55	29	—	8
3	200	43	47	46	—	9
4	200	44	52	31	—	10
5	200	55	47	37	—	13
6	200	40	46	40	—	12
Mean %		25.2	24.6	19.1	17.5	5.4
s		1.8	0.7	1.5	2.7	0.7

Table 2 shows the average results obtained in the examination of each box of fig paste made from each lot of the bulk figs described above. The recovery of the insects and insect heads using cores of 40 to 60 g., and cores of 100 g., are shown separately, expressed as heads per 100 g. paste.

TABLE 2.—*Recovery of heads calculated per 100 g. of fig paste*

BOX NO.	NO. PLUGS PER BOX	LOT 1		LOT 2		LOT 3		LOT 4		LOT 5	
		WT. OF PLUG		WT. OF PLUG		WT. OF PLUG		WT. OF PLUG		WT. OF PLUG	
		40-60g.	100g.	40-60g.	100g.	40-60g.	100g.	40-60g.	100g.	40-60g.	100g.
1	6	2.8	3.0	3.2	2.7	1.7	2.8	1.3	2.0	1.5	0.7
2	6	2.2	1.8	4.0	3.0	1.2	2.5	0.9	1.0	0.5	1.2
3	6	5.1	6.1	5.0	5.3	1.2	2.2	2.0	2.2	0.7	1.0
Mean		3.5		3.9		1.9		1.5		0.9	
F		3.70		1.17		1.33		1.39		2.18	

TABLE 3.—*Relation between filth content of bulk figs and fig paste*

LOT NO.	NO. FIGS EXAMINED	MEAN DEFECTIVE	WT. PASTE EXAMINED	MEAN RECOVERY HEADS PER 100 G. PASTE
		per cent	g.	
1	1200	25.2	2752	3.5
2	1200	24.6	2777	3.9
3	1200	19.1	2857	1.9
4	200	17.5	2739	1.5
5	1200	5.4	3600	0.9

An analysis of variance showed no significant difference between the two core sizes. The mean of the results obtained for both sizes is shown at the bottom of Table 2. The F value for lot 1 was of borderline significance (F at .01 = 3.699) because of the relatively higher recovery in box 3 of this lot. It is seen that the recovery varied from 3.9 to 0.9 insect heads per 100 g. of paste.

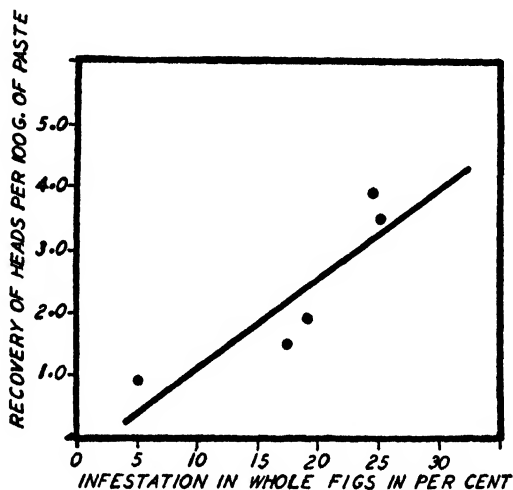


FIG. 1.—The relation between the insect infestation in whole figs in per cent and the recovery of insects and insect heads per 100 grams of fig paste.

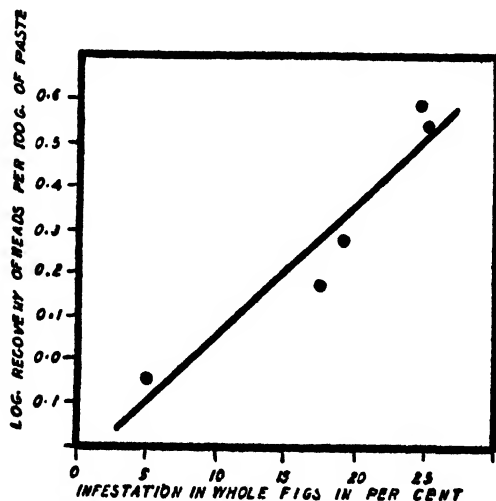


FIG. 2.—The relation between the insect infestation in whole figs in per cent and the logarithmic recovery of insects and insect heads per 100 grams of fig paste.

The mean values obtained for the per cent defective of each lot of bulk figs, in Table 1, and the mean values for the recovery of insects and insect

heads, in Table 2, are combined with the number of whole figs and the weight of paste examined to form Table 3. The data from this table were used to calculate a regression line by the method of least squares, shown in Figure 1. A significant correlation was obtained ($r=0.88$). When the logarithm of the mean recovery per 100 g. of paste was used against the mean per cent defective a highly significant correlation was obtained ($r=0.95$) in Figure 2. On the basis of the latter results it is considered that this is the better correlation to use.

DISCUSSION

The relationship shown in Figures 1 and 2 is deemed to be of considerable value in yielding an estimate of the filth content of fig paste in terms of bulk figs. Growing and handling conditions of this fruit have not advanced sufficiently to produce a product completely free of insects, hence the allowance in commerce of a small amount of filth in this product. In the production of fig paste the insects are broken up and the filth content is more difficult to estimate. Despite the grinding, the recovery of whole insects and heads correlates with the original infestation, and may be used to evaluate the paste in terms of the original figs from which it was made.

SUMMARY

A significant correlation is shown between the amount of insect filth recovered from fig paste and the amount of filth found in the whole figs from which it was made. This correlation provides a quantitative means for assessing filth in fig paste in terms of per cent defective whole figs.

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STEREoisomERIC ANALYSIS OF BETA-CAROTENE*

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Although it has been known for several years that the vitamin A potencies of all-trans- β -carotene, neo- β -carotene-B, and neo- β -carotene-U as measured in experiments on rats (5, 6, 9) stand in the approximate ratios of 6:3:2, respectively, there is still no widely used method of analy-

¹ Now out of print.

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¹ One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

sis for carotene content in foods or feeds which attempts to determine the relative quantities of the stereoisomers present. All-trans- α -carotene is known to have only one-half the vitamin A activity of all-trans- β -carotene (7). In view of the absence of α -carotene in some commodities, such as alfalfa, broccoli, apricots, and sweet potatoes (8, 12, 16), and the predominance of β -carotene in most foodstuffs containing carotene (3, 13), a method for determining the approximate stereoisomer composition of β -carotene should prove useful in obtaining better estimates of vitamin A potency through carotene analysis.

Slowness in adopting methods for evaluating the neo- β -carotene content of carotene extracts has been due to the difficulty of quantitatively separating the closely related stereoisomers. Polgár and Zechmeister (14) showed that the components of an isomerized β -carotene solution could be resolved on a hydrated-lime column with an acetone-hexane mixture as a developer. However, the neo-B zone remained in contact with that of the all-trans form, making it necessary to extrude the column and separate the bands mechanically. The adsorbed pigments were eluted from each of the separate segments with petroleum ether containing alcohol. Kemmerer and Fraps (12) applied this technique to various plant materials. Their method was included in the AOAC collaborative study on carotene in 1945 (11). The difficulty of separating the bands quantitatively led to conflicting results from different laboratories, and it was recommended that the method be further studied and simplified before adoption.

Since approximately 95 per cent of β -carotene occurs as the all-trans, neo-B, and neo-U isomers in an equilibrium mixture (14), work at this Laboratory has been aimed at developing a comparatively rapid, reliable, liquid chromatogram procedure for separating β -carotene extracts into three fractions, each containing primarily only one of these three isomers. Quantitative chromatographic analysis requires careful control of the significant variables such as adsorbent, developer, adsorbate, size of column, and method of packing. In this paper the results of a study of these variables are reported in which the adsorbate was an iodine-isomerized solution of crystalline β -carotene. As a result of this study a procedure is described for the stereoisomeric analysis of β -carotene extracts by liquid chromatogram procedure into neo-B, all-trans, and neo-U fractions, followed by colorimetric analysis of the fractions. The method is sufficiently simple to make its routine application practical. The application of the method to both fresh and dehydrated alfalfa is described in a subsequent publication (3).

STUDY OF VARIABLES IN CHROMATOGRAPHIC SEPARATION

The present study is an extension of earlier work (2) in which various developers were evaluated for their efficiencies in separating an iodine-isomerized petroleum ether solution of crystalline β -carotene into neo-B,

all-trans, and neo-U fractions on a hydrated lime column. Except where otherwise noted, the size of chromatographic tube, the method of packing the column, the adsorbate solution, and the adsorbent were the same as used previously (2). A 1.5 per cent solution of *p*-cresyl methyl ether in petroleum ether (b.p. 88–99°C.) was used as standard developing solution. For each variable studied, these standard conditions were maintained for the other variables.

Adsorbate: The total amount of carotene added to a column is more important than its concentration in determining the degree of separation of the isomers. However, it is preferable to add the desired quantity in a volume of one ml. or less to produce compact, well-defined bands. Our studies showed that increasing the amount of carotene on the column from 20 to 1000 micrograms gives successively poorer separations. Although very small quantities of carotene readily yield good separation of isomers, the resulting bands are quite pale. Thus it is necessary to choose sufficient carotene to produce bands easily visible on the column. About 100 mmg. total carotene (0.2 ml. 0.536 g./l. solution) was used in studying the other chromatographic variables. This quantity produces bands which are easily visible and clearly separated when the standard developing solution is used (2). In the liquid chromatogram procedure given below, 40 mmg. is recommended.

Developers and solvents: Petroleum ether (b.p. 88–99°C.) was used as solvent for most of this work, with some parallel experiments with isooctane. Lower-boiling solvents were unsatisfactory because of excessive volatility. Isooctane has the advantage of uniformity from batch to batch, while petroleum ether usually produced significantly cleaner separations and is less expensive. Occasional lots of petroleum ether were found to be unsuitable because of unknown impurities.

Since earlier work (2) indicated that certain aromatic-aliphatic ethers were superior to the more common developing agents for separating stereoisomers of β -carotene adsorbed on a hydrated-lime column, approximately forty additional compounds of this type were investigated for their influence on the course of the separation of the stereoisomers. None of these was found to be significantly better than the previously investigated compound *p*-cresyl methyl ether, although a number were effective in producing a separation between neo-B and all-trans- β -carotene on the hydrated lime column, especially anisole, phenetole, anethole, β -chloroethyl phenyl ether, *p*-bromo anisole, *p*-, *m*-, and *o*-cresyl ethyl ether, *n*-butyl phenyl ether, phenyl ether, ethyl anisate, α -naphthyl ethyl ether, *p*-methoxydiphenyl, and hydroquinone diethyl ether.

Adsorbent: An adsorbent may be considered suitable for use in a rapid routine, chromatographic procedure if adsorption columns prepared from it are satisfactory with respect to the following properties: rate of permeation of developing solution, rate of development of the column when a

suitable developer is used, separation of bands in the developed column, and visibility of the bands on the column. Since Shell brand hydrated lime,² the adsorbent used in earlier work on separation of β -carotene stereoisomers (2), is no longer commercially available, a survey was made of approximately seventy different samples of available hydrated limes or calcium hydroxides for their suitability for use in this chromatographic

TABLE 1.—*Hydrated lime adsorbents suitable for chromatographic separation of β -carotene stereoisomers*

SOURCE*		
Eimer and Amend Co.	New York, N. Y.	Calcium hydroxide, C.P. Grade
Mallinckrodt Chemical Works	St. Louis, Mo.	Calcium hydroxide, U.S.P. Grade
Kraft Chemical Co.	Chicago, Ill.	Hydrated lime
Mississippi Lime Co.	Ste. Genevieve, Mo.	Mississippi Vertical Combination
Green Mountain Lime Corp.	New Haven Junction, Vt.	Hydrated lime
Baker and Adamson	New York, N. Y.	Reagent calcium hydroxide, Code 1523
Consolidated Chemical Industries	Houston, Texas	Pioneer Hydrated Lime
E. & F. King & Co.	Boston, Mass.	Kemikal Hydrated Lime
Wm. H. Scheel	Brooklyn, N. Y.	Hydrated Lime

* The mention of these products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

procedure. These materials differed greatly in permeation rates and in separating powers, in general showing no correlation between these properties. Approximately two-thirds of the samples showed such low solvent permeation rates that they were not tested further. Over half of the remaining samples produced little or no separation of the isomer zones, required excessively long times for developing the column, or were too dark in color for satisfactory visibility of the bands on the column. The first five products listed in Table 1 were found to be comparable to Shell brand lime for chromatography of the stereoisomers of β -carotene as

² The mention of this product does not imply that it is endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

judged by the criteria listed above. Since only one sample of each product was tested and no attempt was made to make the survey exhaustive, there are doubtless other commercially available products which are equally satisfactory for this purpose. Likewise there is no assurance that other samples of the products listed will perform satisfactorily in this application.

In efforts to prepare adsorbents with superior properties for chromatographic separations, calcium hydroxide was prepared in this laboratory by precipitation from solutions of calcium salts, by hydrating calcium oxide, and by hydrolysis of calcium carbide. Variations of each of these methods yielded products which could be used to separate the stereoisomers of β -carotene, but most of them were inferior to the best commercially available samples. The most satisfactory laboratory product was obtained by slaking reagent-grade calcium oxide with the stoichiometric quantity of water and drying 15 to 30 minutes at 100°C., but successive small batches showed large differences in adsorptive properties. Calcium hydroxide prepared by hydrolysis of calcium carbide was so dark in color as a result of impurities in the carbide that the chromatograph zones were difficult to see, but was otherwise satisfactory. No work was done with adsorbents other than calcium hydroxide.

It was found that the moisture content of hydrated lime is an important factor influencing its adsorptive powers. When samples of Shell brand lime were conditioned in atmospheres varying in relative humidity from 0 to 91 per cent by exposure for 48 hours over saturated salt solutions *in vacuo*, appreciable differences in efficiency of separating the stereoisomers resulted. The optimum range of relative humidity was found to be 30 to 50 per cent. Storage over a desiccant definitely lowered band separation. Heating 16 hours at 100°C. destroyed the separating power, but it was at least partially restored after conditioning in an atmosphere having a relative humidity of 33 per cent. It is thus desirable to store calcium hydroxide used for chromatography in closed containers and to avoid unnecessary exposure of the material to the atmosphere when it is used.

The dependence of the adsorptive properties of calcium hydroxide on both the manner in which it is formed and its treatment subsequent to formation reflects the fundamental roles of particle size and shape, surface area, and surface condition in determining these properties.

Preparation of column: It is desirable that the time required to develop the chromatogram be kept as low as possible, both for economy of time and to minimize the possibility of re-isomerization of any band during the procedure. The development time is determined by the manner in which the column is prepared as well as by the properties of the adsorbent and developing solution. The tightness of packing the column affects both the development time and the degree of separation of the zones. Tightly packed columns usually gave well defined zones, but required 2 to 3

hours for development. Packing the column uniformly under vacuum with a minimum of tamping resulted in more diffuse zones, but the speed of development was increased so that all three major zones could be collected in from 40 to 60 minutes. Columns packed with mixtures of hydrated lime and diatomaceous earth in ratios of 3:1, 2:1, and 1:1 developed increasingly rapidly (15) but were considered unsatisfactory because of diffuseness and resulting low color intensities of the bands.

Increase in packed length of the column increases the separation of the bands but requires longer development times. Analyses by the procedure described below, using 7.5, 10, and 12 cm. effective column lengths, showed a slight difference in results for the first two, but no difference for the last two. The intermediate column length is accordingly recommended. The method of packing routinely employed is described in the following section.

PROCEDURE FOR STEREOISOMERIC ANALYSIS

The following procedure has been found satisfactory for routine stereoisomeric analyses of β -carotene solutions. Twenty-four samples can be analyzed by two experienced operators in an eight-hour day.

Adsorbent: It is recommended that the hydrated lime or calcium hydroxide employed meet the following performance characteristics when an iodine-isomerized petroleum ether solution of crystalline β -carotene is analyzed by this procedure: (1) V_e , the rate of flow of developing solvent through the column when a state of constant flow has been reached, should be at least 2 mm. per minute; (2) the time required to develop the column to the point where the neo-U zone is beginning to leave the column should not exceed 90 minutes; (3) there should be no obvious overlapping of the neo-B and all-trans bands in the developed column. These specifications are rather arbitrary and are intended only as a guide in deciding whether a particular product is suitable for routine analytical use. The products listed in Table 1 easily met these requirements. Columns prepared with the most satisfactory adsorbents clearly separate the neo-B and all-trans bands by at least 5 mm., have flow rates (V_e) of at least 5 mm./min., and require only 30 minutes for the development time mentioned.

Preparation of chromatographic column: The chromatographic tube is 9 mm. inside diameter and 28 cm. in length, with the upper end flared to a diameter of 2.5 cm. to form a convenient funnel for the addition of the adsorbent. The tube is constricted at the lower end and sealed to a capillary tube having a bore of 1 mm. and length of 16 cm. The column is packed to a height of 10 cm., while the pressure is reduced at the lower end to 100 to 200 mm. Hg by means of an aspirator. The adsorbent is added in 5 to 10 separate portions with gentle tapping of the side of the column to obtain uniform packing.

Development of the column: The preparation of the β -carotene extract or

solution to be used for stereoisomeric analysis, as well as the analytical procedure, should be carried out in a darkened room. Two to four foot candles of light intensity have been found adequate for observation of the progress of chromatographic development and cause no detectable isomerization during the time required for the analyses (3, 4).

A sufficient quantity of carotene solution is added to the dry column to give about 40 micrograms of carotene. The chromatogram is developed with a solution of 1.5 per cent para-cresyl methyl ether in petroleum ether (b.p. 88–99°C.). By means of a Fisher Filtrator,² or equivalent, maintained at an absolute pressure of 100 to 200 mm. Hg., the eluate is collected directly in 25-ml. volumetric flasks. The bands are eluted from the column in the following order: neo- β -carotene B, all-trans- β -carotene, neo- β -carotene U. The neo-B fraction contains all the eluate leaving the column before the leading boundary of the all-trans zone reaches the bottom of the column. Further eluate is collected as the all-trans fraction until the leading boundary of the neo-U zone reaches the column outlet. The elution of the neo-U fraction is accelerated by addition to the column of 15 ml. of 5 per cent acetone in petroleum ether when the collection of the all-trans fraction is nearly completed. If colored oxidation or reaction products of β -carotene are present, they remain firmly adsorbed near the top of the column.

Colorimetric analyses: As soon as each fraction is collected, the solution in the flask is made up to volume with petroleum ether and the optical density is determined at once by an Evelyn colorimeter with a 440 m μ filter. If D_b , D_t and D_u are the optical densities of the neo-B, all-trans, and neo-U fractions, respectively, the isomer contents of the three solutions are then given by the equations

$$T = KD_t \quad (1)$$

$$B = 0.78 KD_b \quad (2)$$

$$U = 0.95 KD_u \quad (3)$$

where B , T , U are conveniently expressed in micrograms of isomer present in the fraction. The value of K is determined from measurement of the optical densities of petroleum ether solutions of crystalline (all-trans) β -carotene with the colorimeter and filter to be used for stereoisomeric analyses. The factors 0.78 and 0.95 appearing in the above equations were determined from measurements on solutions of the pure crystalline neo-B, all-trans, and neo-U isomers, the preparation of which was recently described (4, 10). These calibrations have not been checked with other types of colorimeters.

STEREOMERIC ANALYSIS OF AN ISOMERIZED BETA-CAROTENE SOLUTION

Table 2 shows the results of eleven replicate stereoisomeric analyses of a solution of crystalline β -carotene isomerized by iodine in the presence of

ordinary laboratory illumination. These analyses were run on the same day by the above procedure. The apparent concentration of the isomerized solution was determined colorimetrically and 43.7 mmg. were placed on the column for each analysis. In a separate experiment it was found that 1.7 mmg. of the 43.7 mmg. apparent carotene content were accounted for by oxidation or reaction products which were readily separated from the β -carotene on a magnesium oxide column (1). When this is taken into consideration, it is seen from the first column of the table that the recovery

TABLE 2.—*Stereoisomer composition of iodine-isomerized β -carotene solution*

TOTAL CAROTENE RECOVERED	STEREOISOMER COMPOSITION		
	NEO-B	ALL-TRANS	NEO-U
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
42.0	33.3	44.1	22.6
42.4	32.9	44.0	23.1
41.7	32.9	43.8	23.3
42.0	33.2	43.7	23.1
41.6	33.0	44.2	22.8
41.6	31.1	45.1	23.8
41.8	32.4	44.6	23.0
42.4	32.6	43.7	23.7
41.6	33.6	43.6	22.8
42.2	32.8	43.9	23.3
41.6	32.6	44.5	22.9
Average 41.9	32.8	44.1	23.1
σ 0.39	0.65	0.46	0.37

σ = standard deviation from the mean.

of β -carotene isomers from the column was complete within experimental error. The standard deviations shown in the table indicate that the reproducibility of the procedure under favorable circumstances is probably greater than its accuracy, in view of the instability of the neo- β -carotenes. It should also be borne in mind that the fractions as collected in this procedure contain small amounts of the less stable and hence less abundant stereoisomers of β -carotene (14).

The stereoisomer composition of Table 2 can be compared with the chromatographic analysis on hydrated lime of a similar solution by Polgár and Zechmeister (14). They extruded the developed column, carved out the bands, eluted the stereoisomers from the separate zones, and determined the amount of each isomer colorimetrically. Their results are given in Table 3. The first row shows the isomer composition as they reported it, assuming the same extinction coefficient for all the isomers. The second row shows the composition found when the lower extinction coefficients

TABLE 3.—*Stereoisomer composition of iodine-isomerized β -carotene solution (41)*

	NEO-B	ALL-TRANS	NEO-U	NEO-E	LABILE ISOMER
	per cent	per cent	per cent	per cent	per cent
Uncorrected ^a	25	48	22	3	2
Corrected ^b	30	44	21	3	2

^a As given in reference (14), assuming equal extinction coefficients for all isomers.

^b Corrected for differences in extinction coefficients of all-trans, neo-B, and neo-U isomers in accordance with Equations 1, 2, and 3.

of the neo-B and neo-U isomers are taken into account in accordance with the above equations. Since the neo-E and labile isomers are included in the neo-B fraction in our procedure, the results in Tables 2 and 3 are in good agreement.

ACKNOWLEDGMENT

The authors wish to express appreciation to C. R. Thompson and I. V. Ford for assistance on certain phases of this work.

SUMMARY

The variables affecting the chromatographic separation of the stereoisomers of β -carotene on hydrated-lime columns have been studied. As a result of this study, a liquid chromatogram procedure has been developed for stereoisomeric analysis of β -carotene extracts. Three fractions consisting primarily of neo- β -carotene B, all-trans- β -carotene, and neo- β -carotene U are collected separately and analyzed colorimetrically. The method was applied to the analysis of an iodine-isomerized solution of β -carotene. It should be applicable to the stereoisomer analysis of β -carotene extracts from any source.

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DETERMINATION OF BETA-CAROTENE STEREoisOMERS IN ALFALFA*

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Alfalfa meal is bought largely for its β -carotene content and consequent vitamin A potency. The purified carotene fraction of alfalfa meal may be considered to be primarily a mixture of all-trans- β -carotene and its partially cis isomers, since it contains no significant quantity of α -carotene (7, 11). Two of these cis isomers (neo- β -carotene B and neo- β -carotene U) may constitute as much as half of the purified "total carotene" fraction and have considerably lower nutritional value than the all-trans form (4, 5, 6, 9). It is important, therefore, that the relative amounts of these isomers be known.

A convenient chromatographic method for separating β -carotene extracts into three fractions consisting primarily of neo- β -carotene B, all-trans- β -carotene and neo- β -carotene U is described in the preceding paper (3). The application of this method to alfalfa is reported herein and a procedure is outlined for the determination of these stereoisomers of β -carotene in fresh and dehydrated alfalfa.

Influence of light on analytical results: Zechmeister and Polgár showed (12) that all carotenoids tested were photo-labile and stated that the rate of steric change is dependent on the initial configuration. In order to determine if ordinary laboratory illumination would influence the reproducibility of results, a series of analyses were performed on the same meal sample on different days. Between analyses, the meal was stored in the dark under refrigeration. In all cases the "total carotene" fractions were prepared by rehydrating the meal, extracting quantitatively with acetone, transferring to petroleum ether, and passing through a magnesia column (micron brand No. 2642)¹ to remove chlorophyll and xanthophyll prior to separation of the stereoisomers on hydrated lime (Method I). The analytical results on the same meal with uncontrolled light conditions varied widely. In order to ascertain whether this variation was caused by light during the analysis, a meal sample was analyzed both under ordinary light conditions (150–300 foot candles) and under greatly reduced light intensity. Analyses performed under the reduced light (2–4 foot candles)

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¹ Westvaco Chemical Division, Food Machinery and Chemical Co.

yielded higher values for neo-B (Table 1). When the extraction was carried out under reduced light but the chromatography was carried out under ordinary laboratory light conditions, the neo-B value again was lower. In another experiment, the extract was irradiated under an incandescent lamp, followed by chromatography under reduced light. This mixture also yielded lower values for neo-B, in accord with observations that cis isomers

TABLE 1.—*Effect of light on isomerization of carotene in extracts from dehydrated alfalfa meal*

LIGHT CONDITIONS DURING ANALYSIS		STEREoisomer COMPOSITION		
EXTRACTION IN	CHROMATOGRAPHY	NEO- β -CAROTENE B	ALL-TRANS- β -CAROTENE	NEO- β -CAROTENE U
		per cent	per cent	per cent
Daylight	Daylight	19.0	61.5	19.5
		18.0	62.4	19.5
Daylight	Daylight ¹	5.7	71.1	23.2
		8.1	72.5	19.3
Reduced light	Daylight	29.1	52.7	18.1
Reduced light	Reduced light	40.0	44.8	15.2
		42.5	43.4	14.1
Darkness ²	Darkness ²	41.5	43.1	15.4
		42.2	43.8	14.0

¹ 40-minute irradiation of extract at 100 f.c. before chromatography.

² All apparatus covered with dark paper or cloth.

are much more photosensitive than the all-trans form (12). In one experiment the entire analysis was performed in almost complete darkness, with equipment for extraction and chromatography covered with dark paper or cloth. The total isomer contents found for the various experiment of Table 1 were the same. The analytical results agree with those obtained under reduced light (Table 1), indicating that a light intensity of 2–4 foot candles causes no detectable isomerization during the time required for the analysis. Under these conditions, the light intensity, is, however, sufficient to permit adequate observation of the progress of separation of the zones on the column. These experiments demonstrate that light has a very pronounced effect on the isomerization reaction in the total extract prior to chromatography and on the separated isomers after chromatography.

Relation of quantity of carotene extracted to isomer composition: Since light has such a marked influence on the analytical results, it is desirable to keep the manipulations prior to chromatography at a minimum. Elimination of the need for quantitative extraction of the total carotene would greatly simplify the manipulations, since the meal need then only

be soaked in a petroleum ether solution in the dark for a few minutes. An experiment was set up to determine whether partial extraction of the carotene from the meal would result in preferential extraction of any of the isomers. It was found that varying the extraction time over intervals of 15 minutes to 25 hours gave a constant isomer composition with varying amounts of carotene extracted (Table 2). Accordingly, it is not necessary to extract quantitatively the carotene from the meal to determine the isomer composition. Soaking the meal in petroleum ether for 15 minutes gives an extract containing sufficient carotene for stereoisomeric analysis.

TABLE 2.—*Relation of stereoisomer composition to quantity of carotene extracted from alfalfa meal*¹

EXTRACTION PERIOD	STEREISOIMER COMPOSITION			PROPORTION OF TOTAL CAROTENE EXTRACTED ²
	NEO- β -CAROTENE B	ALL-TRANS- β -CAROTENE	NEO- β -CAROTENE U	
(hours)	per cent	per cent	per cent	per cent
$\frac{1}{2}$	39.8	43.0	17.2	47
1	37.2	46.0	16.8	52
4	38.5	44.7	16.8	68
25	41.5	43.2	15.3	84

¹ Cold extraction in dark with petroleum ether.

² Total carotene was determined by the method of Bailey, Atkins, and Bickoff (1).

Effect of xanthophylls and chlorophylls on separation of β -carotene isomers: Another experiment was designed to determine whether petroleum-ether extract obtained by soaking the meal for 15 minutes in the dark could be chromatographed directly on hydrated lime, omitting the preliminary purification to separate the "total carotene" fraction from the chlorophylls and xanthophylls. Several meal samples were analyzed by this rapid, partial extraction procedure (Method II). The same samples were also analyzed by the procedure described above for the "total carotene" fractions (Method I). Similar results were obtained by the two procedures (Table 3).

Fresh whole alfalfa: Eight samples of fresh alfalfa obtained from various localities were analyzed for stereoisomer content by the method given in the procedure for fresh plant materials. Small but significant amounts of neo-B and neo-U were found in all the samples studied (Table 4). Considerable precautions were taken to prevent isomerization between the time the samples were cut and the time they were analyzed. Most samples were frozen immediately after cutting and kept frozen until analyzed. Fresh samples cut from a plot adjacent to the laboratory and analyzed immediately without freezing had the same isomer content as comparable samples taken from the same plot and frozen prior to analysis (Table 4). Several experiments were carried out in darkness, with all equipment

TABLE 3.—*Comparison of two methods of preparing β -carotene extracts for stereoisomeric analysis*

SAMPL	METHOD ¹	STEREISOMER COMPOSITION		
		NEO- β -CAROTENE B	ALL-TRANS- β -CAROTENE	NEO- β -CAROTENE U
Dehydrated No. 1	I	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
		37.1	41.3	21.6
		38.7	40.3	21.0
	II	37.6	39.8	22.6
		37.5	38.9	23.6
Dehydrated No. 2	I	32.1	56.0	11.9
		33.5	55.3	11.1
		33.7	55.2	11.0
	II	31.6	55.3	13.0
		32.0	54.2	13.7
		33.2	53.8	12.9
Sun-cured	I	12.1	76.6	11.3
		12.4	76.5	11.0
	II	13.3	72.2	14.5
		13.0	72.0	15.1

¹ Method I—Quantitative extraction with acetone, followed by preliminary purification on magnesia column prior to chromatography on hydrated lime.

Method II—Partial extraction with petroleum ether, followed by chromatography on hydrated lime.

covered with black paper or cloth. In all cases, neo-U as well as neo-B was found. A sample of commercially obtained crystalline β -carotene prepared from alfalfa contained less cis isomers than the fresh alfalfa. This is to be

TABLE 4.—*Stereoisomer composition of "total carotene" fraction of fresh whole alfalfa*

LOCATION OF FIELD	CONDITION OF SAMPLE	NEO- β -CAROTENE B	STEREISOMER COMPOSITION	
			ALL-TRANS- β -CAROTENE	NEO- β -CAROTENE U
Albany, Calif.	as cut	6.3	84.8	9.0
Albany, Calif.	as cut*	7.1	84.8	8.0
Albany, Calif.	frozen	7.6	83.4	9.0
Albany, Calif.	frozen	7.0	84.7	8.1
Firebaugh, Calif.	frozen	3.0	89.1	7.9
Kerman, Calif.	frozen	4.0	87.6	8.4
Ryer Island, Calif.	frozen	4.6	90.0	5.4
Vorden, Calif.	frozen	8.1	84.6	7.3

* Analyzed in darkness.

expected, since it has been shown that all-trans- β -carotene will crystallize first from a solution containing a mixture of stereoisomers of β -carotene (2).

PREPARATION OF CAROTENE EXTRACTS FROM ALFALFA FOR STEREOISOMERIC ANALYSIS

Dehydrated or sun-cured meal: About 10 g. of meal are soaked in 25 ml. of petroleum ether (b.p. 88° to 99°C.) for 15 minutes in the dark. After filtering, the extract is ready for stereoisomeric analysis as described in the preceding paper (3). For good quality dehydrated meals 1 ml. extract will contain about 40 micrograms of carotene.

Fresh plant material: About 20 g. of fresh or frozen alfalfa is ground in a Waring Blendor² with 200 ml. of acetone for $\frac{1}{2}$ minute. The extract is then filtered rapidly through a coarse filter and added to 25 ml. of petroleum ether in a 500 ml. separatory funnel. The acetone is removed with about 5 separate washes with water and the extract dried over sodium sulfate. After drying it is ready for stereoisomeric analysis.

DISCUSSION OF RESULTS

In accordance with previous work (8) the results reported herein show small but significant amounts of neo-B and neo-U in all samples of fresh alfalfa examined. The amount of cis isomers found in dehydrated meal is much greater than that found in the fresh tissues and demonstrates that much of the stereoisomers present in the meal must have been formed during the dehydration process. The meal samples described in this paper were obtained directly from the dehydrators, with minimum opportunity for change in isomer content.

Some dehydrated meals contained a slightly higher apparent neo-B content than the 33 per cent found in an iodine-catalyzed equilibrium mixture at room temperature (3). This may be due to the formation of higher amounts of the less abundant isomers at the high temperatures required for dehydration. Polgár and Zechmeister (10) found larger amounts of members of the neo- β -carotene A, B, C . . . group to be present in mixtures of the stereoisomers obtained by melting crystals of all-trans- β -carotene than in the equilibrium mixture at room temperature. In our work with alfalfa meal, we have obtained chromatographic evidence of the existence of members of this group other than neo-B. These are included in the neo-B fraction in our analytical procedure.

The results on sun-cured meal and fresh alfalfa (Tables 3 and 4) show that sun-curing produces some cis-isomers at the expense of all-trans. The total carotene content of the sun-cured meal is considerably lower than that of the dehydrated meals.

Nutritional implications: Neo- β -carotene B has 53 per cent of the

² The mention of these products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

growth-promoting activity of all-trans- β -carotene in rats (5) or chicks (6), and neo- β -carotene U is variously reported to have 25 per cent (9) or 38 per cent (4) of the activity of all-trans- β -carotene when fed to rats. Most estimates of the vitamin A equivalence of carotene in dehydrated meal are based on the assumption that all the β -carotene is in the all-trans form. Data in this paper show that as much as 60 per cent of the carotene may exist in partially cis forms. If the corrections mentioned above are applied to a commercial meal containing 38 per cent neo-B, 41 per cent all-trans, and 21 per cent neo-U, the calculated nutritional value will be 30 per cent lower than found by a "total carotene" analysis, which assumes an isomer composition of 100 per cent all-trans- β -carotene.

SUMMARY

The liquid chromatogram procedure has been applied to the stereoisomeric analysis of β -carotene extracts of alfalfa into three fractions consisting primarily of neo- β -carotene B, neo- β -carotene U, and all-trans- β -carotene. The presence of light during extraction and analysis will cause isomerization of the pigments. In reduced light this source of error is minimized.

Neo- β -carotene B and neo- β -carotene U may constitute as much as one-half of the β -carotene content of dehydrated alfalfa meal. If no correction is made for them, analyses which yield only "total carotene" values will indicate vitamin A potencies as much as 30 per cent higher than those in which due allowance is made for the reduced nutritional value of these stereoisomers.

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DETERMINATION OF DDT IN SOILS

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In one of the treating procedures authorized as a basis for certification of plants to be shipped from areas regulated by the Japanese beetle quarantine, DDT is applied at the rate of 25 pounds per acre and cultivated into the soil to a depth of 3 inches (1). Owing to cultural practices, retreatment is often necessary when analyses of soil samples taken from treated areas indicate a loss in DDT content. The method described was developed for the purpose of simplifying the work of annual adjustment of nursery soils to this prescribed level. The program involved the sampling of a large number of plots of various soil types and provided only a short period of time for analysis.

METHOD OF ANALYSIS

The samples are composites of 50 borings, each 2 inches in diameter and 3 inches in depth (471.24 cubic inches (V)²) and taken at locations rather evenly spaced throughout areas of 20,000 square feet or less. Sampling is best accomplished when the soil is moist.

PREPARATION OF SAMPLE

Brush the composite sample thru a 4-mesh screen, mix thoroly, and weigh (W_1).³ Pour the sample into a can of such dimensions that the soil is at least 8 inches deep. Subsample by means of a thin-walled steel tube 1 inch in diam., taking seven cores, one from the center and one two-thirds the distance from the center to the rim on each of six radii equally spaced around the can. Weigh the subsample (W_2),³ expose in shallow trays until nearly air-dry, reweigh (W_1),³ and store in airtight jars pending analysis. If duplicate subsamples are required, shake the can to close the holes left by the subsampler and take another subsample in the same way.

SPECIAL APPARATUS

- (1) *Extraction jars*.—Quart Mason jars fitted with glass or plastic-lined lids.
- (2) *Shaking machine*.—A machine that rotates the jars end over end about 25 times per minute.

- (3) *Electrometric titrimeter*.

- (4) *Electrode system*:

- (a) *Silver electrode*.—Solder insulated copper wire to one end of a 2-inch length of 10-gauge pure silver wire. Seal into a 6-inch length of glass tubing with De Khotinsky cement so that $\frac{3}{4}$ inch of the silver wire is exposed. Connect the other end of the copper wire to the electrometric titrimeter. Clean this electrode occasionally by gently rubbing the silver surface with fine emery cloth.

- (b) *Mercury-mercurous sulfate electrode* (Fig. 1).—Pour enough electrolytic mercury (A) into a wide-mouth 4-ounce jar (B) to make a layer 3 to 5 mm deep, and

¹ Grateful acknowledgment is made to S. R. Dutky, for his suggestions on the mercury-mercurous sulfate electrode, and to L. B. Parker for the preparation of the photograph.

² Term used in formula for calculating results.

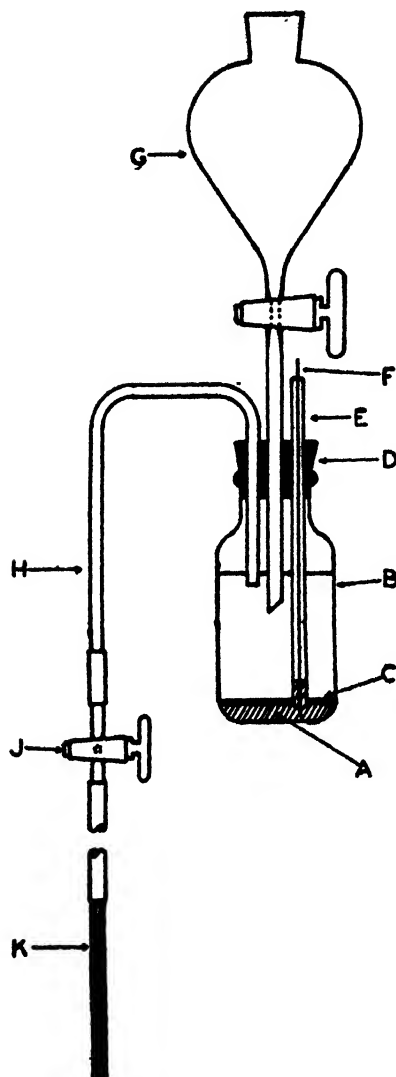


FIG. 1.—Mercury-mercurous sulfate electrode.

REAGENTS

Benzene.—Chlorine and thiophene free.

Isopropanol.—99%.

Metallic sodium.—A.C.S. grade.

Hydrogen peroxide.—30% reagent grade.

Bromthymol blue.—0.4% soln.

Sulfuric acid.—A.C.S. grade.

Celite.—C.P. analytical filter aid.

Silver nitrate soln (0.025 N).

Potassium thiocyanate soln (0.025 N).

then add a paste (*C*) made from a small amount of mercurous sulfate and 0.1 *N* sulfuric acid. Allow the paste to settle and fill the jar three-quarters full with the acid. Close the jar with a three-hole rubber stopper (*D*). Thru one hole pass a glass tube (*E*) with a sealed-in platinum wire tip until contact with the mercury layer is made. Join the other end of the platinum wire to a copper wire (*F*) by means of a mercury seal. Connect the other end of the copper wire to the electrometric titrimer. Thru another hole pass the stem of a separatory funnel (*G*) until its tip is below the surface of the acid in the jar. Thru the third hole pass one end of a glass siphon tube (*H*) until the end in the jar is below the level of the acid. To the other end connect a stopcock (*J*). Apply narrow bands of lubricant to the ends of the stopcock plug to provide for a continuous acid film at all times. Connect a capillary tube (*K*) 6 inches long to the other end of the stopcock. Mount the electrode above the soil to be titrated, partially fill the separatory funnel (*G*) with 0.1 *N* sulfuric acid, and fill the siphon (*H*). Flush the capillary tube (*K*) by opening stopcock *J* for a few seconds and insert into the solution to a depth of about 2 inches.

DETERMINATION

Weigh 300 g of the subsample and pipette 300 ml of extracting solvent (benzene 2 plus isopropanol 1) into an extraction jar, seal, and place on the shaking machine. Remove after $\frac{1}{2}$ hour of agitation and filter into a suction flask thru a Büchner funnel, using gentle suction. Transfer a 200-ml. aliquant into a 500-ml Erlenmeyer flask and evaporate almost to dryness on a water bath kept close to 90°C. Add 25 ml of isopropanol and 2.5 g of metallic sodium cut into small pieces. Reflux for at least $\frac{1}{2}$ hour on a hot plate, shaking occasionally. Eliminate the undecomposed sodium by cautiously adding thru the condenser 20 ml of dilute (1+1) isopropanol. Boil for a few minutes, add 25 ml of water, cool, add 5 ml of hydrogen peroxide 1 ml at a time, and boil for 15 minutes. Disconnect the flask from the condenser, cool, add a few drops of bromthymol blue, neutralize with dilute (1+2) sulfuric acid, add 5 ml of the acid in excess, and cool. Add an excess of 0.025 *N* silver nitrate, coagulate the precipitated silver chloride, add about 1 g of celite, and filter thru a fast quantitative paper into a 400-ml beaker. Wash flask, paper, and precipitate thoroly with small portions of water, keeping the filtrate and washings between 250 and 300 ml. Titrate the excess silver nitrate in the filtrate with 0.025 *N* potassium thiocyanate. Use an electrometric titrimer equipped with silver and mercury-mercurous sulfate electrodes. Between titrations wash the electrodes with water and flush the capillary tip. Conduct a reagent blank and, whenever possible, soil blanks at the same time and in the same manner.

CALCULATION OF RESULTS

Subtract the quantity of silver nitrate found in the filtrate from that originally added. The difference (corrected for the blank) will be that required to combine with the chlorine liberated from the DDT. The weight of chlorine times 2 will give the amount of DDT in the aliquant titrated (*U*).^a Calculate the DDT as pounds per 3-inch acre by the following formula, rounding to the nearest $\frac{1}{2}$ pound:

$$\text{Pounds per 3-inch acre} = \frac{U \times W_1 \times W_2 \times 41,486}{S \times W_2 \times V}$$

Where *U* = Weight of DDT in aliquant titrated (grams).

S = Weight of nearly air-dry subsample represented by aliquant (grams).

*W*₁ = Weight of nearly air-dry subsample (grams).

W_1 = Weight of subsample taken from screened composite sample (grams).

W_2 = Weight of screened composite (grams).

V = Volume of composite = Top area \times depth of boring \times number of borings (cubic inches).

41,486 = Factor to convert grams per cubic inch to pounds per 3-inch acre.

DISCUSSION

The method is based on the determination of total organic chlorine as described by Umhoefer (2). Though not specific for DDT, his method has been adapted to the determination of DDT in agricultural sprays, spray residues, and various foods (3, 4, 5, 6). The colorimetric method of Schechter *et al.* (7) was found to be too time-consuming for routine work. The color bodies extracted from soils interfered with the method of Stiff and Castillo (8).

It is conceivable that chlorine-bearing decomposition products of DDT may accumulate in the soil from year to year and eventually vitiate results by the present method, but there is no evidence at this time that such is the case. Final judgment must be based upon critical studies of the ultimate fate of DDT in various types of soil, using more specific chemical (or biological) methods.

Laboratory-prepared mixtures of DDT and a sandy loam, a clay soil, and a muck soil were used in developing the method. These soils were selected as being representative of soils that might be encountered in nurseries. It was found that the recovery of DDT from these mixtures was influenced by such variables as the extracting solvent, the moisture content, and the type of soil.

The recovery of DDT from these mixtures was best when they were extracted for 30 minutes with a mixture of 2 parts by volume of benzene and 1 part of 99 per cent isopropanol. Several investigators (3, 5, 9) have used benzene as the solvent to remove DDT from spray residues. However, when this extractant was used, the recoveries from a sandy-loam mixture prepared at the rate of 50 pounds of DDT per 3-inch acre were 49, 41, 35.5, 36.5, and 34.5 pounds when analyzed immediately, 1 week, 2, 5, and 7 months after preparation, respectively. From a sandy-loam mixture prepared at the rate of 25 pounds of DDT per 3-inch acre, analyzed 7 months after preparation with petroleum ether, acetone, ethanol, or a mixture of benzene and acetone as the extractant, the recoveries were all about 17 pounds. With benzene and isopropanol, 2+1 parts by volume, as the extractant, close to theoretical recoveries were obtained immediately after preparation and periodically over a period of 2 years.

Benzene and isopropanol mixed 2+1 parts by volume (2.2+1 parts by weight) approximates very closely the azeotropic mixture of these compounds. That mixture, 2+1 parts by weight, has a boiling point of 71.9°C., which is considerably lower than the boiling points of its constituents.

The possibility of decomposing the DDT by overheating while the solvent is evaporating, as suggested by Fleck (4) and others (5, 10), is thus reduced. When a soil extract to which DDT had been added was evaporated in a water bath kept close to 90° C., the recovery of DDT was 99.6 per cent. A similar recovery was obtained when the residue was allowed to remain in the water bath 1 hour after evaporation of the solvent was complete.

Electrometric titration is used because color retained in the treated extract from most soils obscures the end point of a visual titration.

The silver and mercury-mercurous sulfate electrodes are easily prepared and maintained. The silver, or indicating, electrode is not attacked by the solution acidified with sulfuric acid. In the reference electrode the concentration of the mercurous ions in contact with the mercury is kept constant by covering the mercury with a suspension of slightly soluble mercurous sulfate in dilute sulfuric acid, thus providing a common anion in both the salt and the solution (11).

Back titration with potassium thiocyanate has been found to be more sensitive and consequently preferable to direct titration with silver nitrate for the analysis of miscellaneous soils. One-tenth ml. of 0.025 *N* potassium thiocyanate gives a potential change near the equivalence point of about 35 millivolts, as compared with about 10 millivolts by direct titration with silver nitrate of the same normality. Thus a sharp end point is obtained more rapidly by back titration. However, this procedure is a little longer than the direct procedure. Since certain soils contain a considerable amount of extractable material, both procedures involve filtration. Direct titration in the presence of this material may result in the fouling of the silver electrode. The additional time required for precipitation in the back-titration procedure is partly compensated for by the time saved in titrating.

The average recoveries of DDT, rounded to the nearest $\frac{1}{2}$ pound, from laboratory-prepared mixtures of DDT with several soils of variable moisture content are shown in Table 1.

TABLE 1.—Average recovery of DDT, in pounds per 3-inch acre, from several soil mixtures prepared at the rate of 25 pounds of DDT per 3-inch acre

CONDITION OF SOIL MIXTURE WHEN ANALYZED	SANDY LOAM		CLAY SOIL		MUCK SOIL	
Air-dry	22	(10)*	24	(6)	26	(6)
Nearly air-dry	24	(30)	23	(10)	23.5	(16)
Just below maximum water-holding capacity	21	(2)	19	(2)	21	(2)

* Figures in parentheses are the number of determinations made.

With air-dry soils the recoveries were a little higher in the clay and muck soils and lower in the sandy loam than in the nearly air-dry soils. With the wet soils the recoveries were considerably lower. The variation between the various soils was least when the soils were nearly air-dry.

These recoveries were obtained by subtracting soil and reagent blanks from the total DDT determined. Since in many cases it is impossible to secure untreated soils for blanks, the results were recalculated by subtracting the reagent blank only. The soil blanks were 1, 1, and 3.5 pounds for the sandy loam, clay soil, and muck soils, respectively. The reagent blank was 1.5 pounds. The recalculated recoveries from the nearly air-dry soils were 25, 24, and 27 pounds DDT per 3-inch acre for the respective sandy, clay, and muck soil-DDT mixtures.

A total of 84 samples taken from nursery plots treated with DDT were analyzed. The differences between analyses on duplicate subsamples from the same composite averaged 1 pound per 3-inch acre, and ranged between 0 and 2.5 pounds.

This method has also been adapted to the determination of DDT in spray and dust residues on apples, peaches, potatoes, elm twigs, foliage, paper toweling, and glass plates.

SUMMARY

A method for the determination of DDT in soil is given. The average recoveries from sandy-loam, clay-soil, and muck-soil mixtures prepared at the rate of 25 pounds DDT per 3-inch acre were 24, 23, and 23.5 pounds, respectively. The recoveries from the same mixtures were 25, 24, and 27 pounds when no soil blanks were subtracted, since in many cases it is impossible to secure untreated soils. The variation between soils was least when analysis was made when the soils were nearly air-dry. The differences between recoveries from duplicate subsamples averaged 1 pound per 3-inch acre and ranged between 0 and 2.5 pounds. The method has been successfully used for the determination of DDT in spray and dust residues. The description of a convenient dip-type electrode is given.

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MICROSCOPIC IDENTIFICATION OF SUCCINIC ACID AS BARIUM SUCCINATE

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In a paper³ on the determination and identification of lactic and succinic acids in foods, the method for succinic acid there given directs that, after the final titration with barium hydroxide, the aqueous layer be evaporated



FIG. 1.—Barium succinate crystallized from water.

until crystals appear and that these be compared with crystals of pure barium succinate similarly prepared. However, the microscopic-crystallographic constants of barium succinate crystals were not given at that time.

These measurements have been made on crystals obtained from decomposed eggs, and were found to be identical in every respect with those of pure barium succinate. In both cases the aqueous solution of barium succinate was evaporated almost to dryness and allowed to stand until the

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³ Clayborn and Patterson, *This Journal*, 31, 134 (1948).

crystals formed. The crystals were then removed to a microscopic slide, dried, and examined under the polarizing microscope by the immersion method.

MICROSCOPIC-CRYSTALLOGRAPHIC PROPERTIES OF BARIUM SUCCINATE

Barium succinate crystallizes from water as bipyramids and prisms of the tetragonal system, having a tabular and long prismatic habit (see Fig. 1). In parallel polarized light (crossed nicol prisms) the crystals exhibit parallel and symmetrical extinction. Elongated prisms exhibit extinction parallel to the long c axis and positive elongation. In convergent polarized light a positive uniaxial interference figure slightly inclined to the optic axis is observed on tabular bipyramidal crystals and on crushed particles which do not extinguish sharply. The principal refractive indices are $n_o = 1.580$ and $n_e = 1.633$, both ± 0.002 . The index of the ϵ ray may be measured on the long axis of the elongated prisms and on crushed particles showing maximum birefringence.

DETERMINATION OF MONOFLUOROACETIC ACID IN FOODS AND BIOLOGICAL MATERIALS*

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Sodium monofluoroacetate, also known as 1080, is finding increasingly widespread use as a rodenticide. Because of its extreme toxicity to all warm-blooded species, the use of 1080 by commercial exterminators in food warehouses and manufacturing plants presents problems of grave concern to health and regulatory officials. Hence, in regulatory and pharmacological work there is urgent need for a method for determining micro amounts of monofluoroacetic acid in foods and biological materials.

The method proposed here for the determination of monofluoroacetic acid is based upon the fluorine content of an isolated fraction containing only organic acids. The essential steps of the method are:

- (1) Sample preparation, which includes a protein separation step in case of protein-containing materials and may include a hot aqueous extraction or an enzymatic digestion in certain instances,
- (2) Ether extraction, followed by removal of all acids from the ether with aqueous alkali,
- (3) Separation of fluoroacetate from inorganic fluorine compounds by partition chromatography, employing a silicic acid column with 0.5 N

* Presented in part at the meeting of the Washington Section of the American Chemical Society May 12, 1949. Essentials of the method in mimeographed form have been distributed to regulatory officials and other interested persons.

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sulfuric acid as the immobile solvent and chloroform containing 10 per cent by volume of tertiary amyl alcohol or *n*-butyl alcohol as the mobile solvent, and

(4) Ignition of the fluoroacetic acid with lime to convert the fluorine to inorganic fluoride, which is then determined by an established technique (1).

The method is highly specific for monofluoroacetic acid. Although inorganic fluorides or fluosilicates are readily extracted from an acid solution with ether, a 1000-fold excess of mineral fluoride does not materially affect the analysis. The critical step in the procedure is the chromatography, in which the separation of monofluoroacetic acid from inorganic acids containing fluorine is 100% complete. Since the percolate from the chromatographic column contains only organic acids, only fluorine-containing organic acids are measured. No such acids are known to exist naturally in foods or biological tissue,¹ but the method would measure the fluorine of difluoroacetic and trifluoroacetic acids, if present. Apparently these acids do not occur in commercial 1080 to any appreciable extent, as indicated by a total fluorine analysis.

Sensitivity of the procedure is governed by that of the micro-method for the determination of fluorine. With careful evaluation and stability of the reagent and apparatus blank (about 5 micrograms F), as little as 10 micrograms F can be determined with good precision. This quantity of organic F represents about 41 micrograms of monofluoroacetic acid. With a 200-gram sample containing 41 micrograms of fluoroacetic acid in the aliquot taken for titration, this permits a quantitative determination at a level of about 0.2 p.p.m.

A colorimetric microanalytical method for acetate and fluoroacetate, based upon the lanthanum nitrate-iodine qualitative test for the acetate ion, has been proposed by Hutchens and Kass (2). Besides lacking specificity their method failed to recover fluoroacetate added to grain and tissue; furthermore, they were unable to recover the fluorine of the added 1080.

Our findings by the method presented here do not confirm those of Hutchens and Kass and do not support their suggestion that the fluoroacetate is firmly bound or converted to another substance. We have found that fluoroacetic acid added as the potassium salt to biological tissue and a wide variety of foods can be recovered essentially quantitatively. However, in order to obtain reproducible quantitative recoveries of added fluoroacetate we did find it necessary to subject flour samples to a pancreatic digestion, when the fluoroacetate had been added in aqueous solution and the flour air dried for a period of several weeks prior to analysis. Furthermore, it has been found that a pancreatic digestion,

¹ A South African plant, Gifblaar, *Dichopetalum Cymosum*, however, has been found to contain monofluoroacetic acid. (*Onderstepoort Journal of Veterinary Science and Animal Industry*, 20, 67 (1944).)

or even a pre-cooking of the sample in boiling water, will improve the recoveries of 1080 from poisoned rats.

METHOD

APPARATUS

- (1) Chromatographic tubes, 18 mm O.D. \times 250 mm long, prepared from Pyrex tubing.
- (2) Suitable pressure source, such as compressed air or a cylinder of nitrogen or carbon dioxide; and a means of keeping the pressure constant, such as a column of mercury or a diaphragm type pressure regulator.
- (3) Mixer of the Waring Blendor type.
- (4) Fluorine apparatus listed in *Methods of Analysis*, A.O.A.C., Sixth Ed., sec. 29.24.

REAGENTS

- (1) *Silicic acid*.³
- (2) *Mobile solvent*. Add 100 ml of tertiary amyl alcohol⁴ or *n*-butyl alcohol to 900 ml of U.S.P. chloroform and mix.
- (3) *Sulfuric acid solns*: 1+1, ca 0.1 *N* and ca 0.5 *N*.
- (4) *Sodium hydroxide solns*, ca 1.0 *N* and ca 0.1 *N*.
- (5) *Phenolphthalein soln*, 1%.
- (6) *Trisodium phosphate soln*, saturated.
- (7) *Pancreatin*, U.S.P.
- (8) *Ether*, A.C.S.
- (9) *Phosphotungstic acid*, 20% *W/V*.
- (10) Fluorine reagents listed in *Methods of Analysis*, A.O.A.C., Sixth Ed., sec. 29.25.

PROCEDURE

(1) *Preparation of sample*.—This will vary with different types of materials. Sugar can merely be dissolved in water, acidified with sulfuric acid, and extracted directly. A simple water wash may be adequate to prove contamination of certain other foods.

(a) *Sugar*.—Dissolve 100 g of sugar in sufficient water to give a volume of ca 350 ml.

(b) *Flour*.—Place 100 g of flour in the mixer, add 400 ml of water and 5 g of pancreatin, and comminute ca 2 minutes. Adjust to pH 7–8, using the saturated soln of Na_2PO_4 and a suitable indicator paper.⁴ Transfer the comminuted material to a tared 1-liter Erlenmeyer flask, washing the mixer three times with 25 ml portions of water. Incubate the mixture at 35–40°C. for at least 3 hours. Add 5 ml of (1+1) H_2SO_4 and swirl. Add 20 ml of 20% *W/V* phosphotungstic acid and swirl again. Make up to 750 g with water, stopper, and shake vigorously ca 2 minutes. Filter thru a Büchner funnel (16 cm size is convenient) with suction or thru a fluted filter. Use at least 375 g aliquot of filtrate. (Since the specific gravity of the filtrate is very close to 1, measuring out the aliquot in a graduated cylinder is satisfactory.) If only small continuous extractors (less than 400 ml capacity) are available, neutralize the filtrate aliquot to phenolphthalein with strong NaOH and finally adjust the soln just to the alkaline color of the indicator with the 0.5 *N* H_2SO_4 and *N* NaOH. Concentrate the soln to a volume of ca 100 ml by boiling. If the soln bumps or spatters while boiling, place on a steam bath; a current of air will hasten the evaporation.

³ Mallinckrodt's Analytical Reagent Grade precipitated powder was used in this work.

⁴ Sharples Chemicals, Inc. Technical Product was used.

⁵ Fisher Alkacid test paper is satisfactory.

(c) *Wheat*.—Grind finely in a suitable mill such as a Wiley mill. Proceed as for flour.

(d) *Corn meal*.—Proceed as for flour in (b) above, except omit the pancreatic digestion.

(e) *Corn*.—Grind and proceed as for corn meal.

(f) *Peanuts*.—Grind finely (like peanut butter) and proceed as for cornmeal, except use 100 ml of the 20% phosphotungstic acid per 100 g sample. It may be necessary to refilter thru a folded filter to remove oil.

(g) *Cheese*.—Proceed as for cornmeal, except use 40 ml of phosphotungstic acid for the 100 g sample.

(h) Other foods such as chili peppers, cacao beans, etc., can be treated in a manner similar to the above.

(i) *Biological tissue*.—If the material is tough or fibrous, grind it twice thru a food chopper. (Soft tissues, e.g. brain and liver, need not be ground.) Place 100 g of the ground tissue in an 800 ml beaker, add ca 300 ml of water, cover with a watch-glass, and cook by boiling gently ca one-half hour. Transfer the material to the mixer, rinsing the beaker with two 25 ml portions of water, and comminute thoroly (ca 2 minutes). Transfer the comminuted material to a tared liter Erlenmeyer flask, rinsing the mixer with two 25 ml portions of water. Add 5 ml of the 1+1 H_2SO_4 and mix. Add sufficient 20% phosphotungstic acid (usually 50–75 ml) to precipitate all the proteins, then water to make the weight 600 g. Shake vigorously for ca 2 minutes and filter thru a fluted filter paper, or with suction thru a Büchner funnel. If the material does not filter rapidly, return the mixture to the flask, add ca 10 ml more of the phosphotungstic acid, shake vigorously, and refilter.

Alternative procedure to cooking the tissue: Place 100 g of ground tissue in the mixer, add 300 ml water and 15 g of pancreatin, and comminute thoroly (ca 2 minutes). Adjust the pH to ca 8 with the saturated Na_3PO_4 soln using a suitable indicator paper. Transfer the comminuted material from the mixer to a tared liter Erlenmeyer flask, washing the mixer with two 25 ml portions of water; and incubate ca 3 hours at 35–40°C. Continue by precipitating the proteins and making to volume as above.

(2) *Ether extraction*.—Transfer the soln in the case of sugar or a weight-aliquot of the protein-free filtrate (in the case of protein-containing materials) to a large continuous extractor (200 ml size described in *Methods of Analysis*, A.O.A.C., 26.45 (b); 1500 ml extractors of this type have been used successfully. An extra coarse fritted filter tip^a on the bottom end of the inner tube aids in getting smaller droplets of extracting solvent). For each 50 grams of soln, add 1 ml of H_2SO_4 (1+1). Extract with ether until all fluoroacetic acid has been extracted, as determined by a preliminary experiment (usually 3–4 hours with a 400 ml extractor). Transfer the ether extract to a separatory funnel of appropriate size.

To the extraction flask add ca 20 ml of water, two drops of 1% phenolphthalein indicator soln, and sufficient 1.0 N NaOH from a buret to give the strong alkaline color of the indicator after swirling. Pour the rinse soln into the separatory funnel and add more alkali until the alkaline color of the indicator persists in the aqueous phase after vigorous shaking. Record the volume of alkali required. Draw off the aqueous layer into a 100-ml beaker and wash the ether with two 10-ml portions of water, rinsing the extraction flask each time with the water before pouring it into the separatory funnel. Add the washings to the beaker. Carefully adjust the alkalinity of the extract just to the alkaline color of phenolphthalein with 0.1 N H_2SO_4 and NaOH solns. Evaporate the neutralized extract to dryness on the steam bath.

^a Ace Glass Inc. Cat. No. 8600, porosity A is suitable.

(A current of air will hasten the evaporation.) If during the evaporation the alkaline color of the indicator should disappear, add just sufficient 0.1 *N* NaOH to give the alkaline color again. Do not continue heating after the residue is apparently dry. (One can continue with a residue which is slightly moist.)

(3) *Chromatography*.—To 5 g of silicic acid in a mortar add the maximum amount of 0.5 *N* H₂SO₄ that it will hold without becoming "sticky" (ca 50–80% of its weight). Mix well with the pestle, then add ca 35 ml of the mobile solvent and work up into a slurry. (This should be smooth; if the silica agglomerates in the solvent, too much 0.5 *N* H₂SO₄ was used.) Place a small cotton plug in the bottom of a chromatographic tube and pour in slurry tilting the tube slightly to avoid air bubbles. Allow the silicic acid to pack down under 2–10 pounds pressure applied thru a gas pressure regulator. When excess solvent has drained thru (column firm and viscous enough to resist pouring when tipped), the column is ready for use. (In this step, care must be exercised to avoid cracking or drying out of the gel; this is caused by leaving the pressure on after the column has packed down and all the solvent has sunk into the gel. However, if a column does crack, the determination need not necessarily be lost even though the sample has been added to the column. Rejuvenate the column by removing the Na₂SO₄, adding ca 10 ml of the mobile solvent to the gel, and stirring it thoroly with a long glass rod. Allow the column to repack under pressure, return the Na₂SO₄ to the column, fill the tube up with solvent, and then collect sufficient percolate from this point, to obtain all the fluoroacetic acid when combined with that which had passed thru prior to the cracking, if any.)

To the dry or slightly moist residue in the 100-ml beaker add sufficient 1+1 H₂SO₄ (ca 18 *N*), usually 0.5–1.0 ml, to give an excess of ca 0.25 ml of (1+1) H₂SO₄ over the amount calculated as being required to convert all the salts to the free acid, based upon the amount of *N* NaOH required to neutralize the acid extracted by the ether. Wet the salts *thoroly* with the acid, using a small narrow blade spatula (steel or monel metal) to loosen the salts from the glass and using a glass tamping rod (stirring rod flattened on one end) to break up the solid particles and mix the resulting slurry. Add anhydrous granular Na₂SO₄, 5–10 g usually being sufficient, to take up the excess liquid. Stir well with the tamping rod, breaking up any lumps that may form. Add 10 ml of the mobile solvent, stir thoroly, and decant the solvent carefully onto the column.

Place a graduated cylinder under the column to catch the percolate and apply pressure. When all the solvent has sunk into the gel, release the pressure. Add 5 ml of the mobile solvent to the beaker and again stir thoroly. Decant the solvent carefully onto the column and with the aid of a narrow blade spatula, transfer the bulk of the material in the beaker, mostly Na₂SO₄, to the column. Renew the pressure. When the solvent has passed ca halfway thru the Na₂SO₄, release the pressure. Rinse out the beaker with another 5 ml portion of solvent and transfer to the column. After this washing has sunk ca half-way into the sodium sulfate, fill the tube with the mobile solvent and complete the collection, under pressure, of sufficient percolate, dropwise (3–4 ml per minute is a convenient rate), to obtain all the fluoroacetic acid as determined by a preliminary experiment. (With several batches of silicic acid this amount of percolate was found to be 50 ml.)

Transfer the percolate to a 125 ml separatory funnel, add ca 20 ml of water and sufficient 1.0 *N* NaOH to give the alkaline color of phenolphthalein (present in percolate; no additional phenolphthalein required) in the aqueous phase, after vigorous shaking. Draw off the aqueous layer into a 125-ml Erlenmeyer flask and return the solvent layer to the separatory funnel. Wash the solvent twice with 10 ml portions of water and add the washings to the Erlenmeyer flask. Aerate the soln with a current of air to remove traces of chloroform. (If excess chloroform is not removed, excessive chloride may complicate the F distillation in step 4.)

(4) *Determination of fluorine*.—Transfer the aqueous extract to a platinum dish with a little water, mix with ca 20 ml of the lime suspension (*Methods of Analysis*, A.O.A.C., 29.25,) evaporate to dryness, and ash briefly (15–20 minutes) at 600°C. (A little carbon in the ash will not interfere in the determination.) Proceed as directed under 29.26 (a), 29.27, 29.28. (Use of the 100-ml size Nessler tubes is preferable.) Convert F results to fluoroacetic acid or to sodium monofluoroacetate (1080) as desired and correct for the aliquot taken, if any, in the extraction step. The volume occupied by the insoluble solids is ignored.

RESULTS AND DISCUSSION

Analysis of commercial sodium monofluoroacetate (1 lot) for fluorine gave 91 and 94% (duplicate determinations) of the theoretical value. This material contained 0.9% chlorine.

Since the fluorine content of commercial 1080 was somewhat low and an appreciable amount of chlorine was present, the product was purified for the experimental work. The commercial 1080 was dissolved in water, acidified with an equivalent amount of sulfuric acid, extracted in a large continuous extractor with ether, and the ether distilled off. The fluoroacetic acid was distilled and refractionated, the fraction boiling at 167–167.5°C. being taken. This fraction was dissolved in alcohol, neutralized with alcoholic potassium hydroxide, and the precipitated potassium salt recrystallized from alcohol. Fluorine in the potassium salt was found to be 16.1 and 16.2% (duplicate determinations); the theoretical value is 16.36%. This salt was deemed to be pure enough for our purpose; in fact, it was considered 100% pure in calculating the recovery experiments. The pure potassium salt as well as commercial 1080 was found to be appreciably hygroscopic when the atmospheric humidity was above a certain undetermined level.

Step two of the method directs that the aqueous extract containing the fluoroacetic acid be neutralized just to the phenolphthalein end point and evaporated to apparent dryness and that heating is not to be continued after this point. Prolonged heating after apparent dryness has been reached always leads to low results, regardless of the pH at which the solution was adjusted prior to the evaporation. If the pH is too high, there appears to be some hydrolysis and if the pH is too low, the loss appears to be due to volatilization of the acid. Table 1 shows the effect on fluoroacetic acid recovery by evaporating its solution at various pH levels and drying the residue for various periods of time at 100°C. in preparation for the chromatography step. In these experiments, solutions of potassium fluoroacetate equivalent to 1 mg of fluoroacetic acid were used. It will be noted from the table that evaporating a solution of the pure potassium monofluoroacetate in the presence of ca 0.7 g of Na_2SO_4 , and heating the residue overnight, resulted in a low recovery, 70%.

An experiment was conducted to determine the ease of hydrolysis of fluoroacetic acid. One milligram of fluoroacetic acid in the form of its potassium salt was added to 25 ml of *N* NaOH and placed on a steam bath

TABLE 1.—*Effect of pH and heat on recoveries of monofluoroacetic acid*

pH	PERIOD OF HEATING	RECOVERY
		per cent
1. Alkaline color of phenolphthalein	Just to dryness	96, 98
2. Alkaline color of methyl red	About 5 min. after apparent dryness	90
3. Alkaline color of bromocresol green	About 5 min. after apparent dryness	81
4. pH7 (borax-phosphate buffer)	Overnight—about 16 hours	66
5. pH8 (borax-phosphate buffer)	Overnight—about 16 hours	56
6. Alkaline color of phenolphthalein	Overnight—about 16 hours	77, 79
7. Soln containing 0.7 g of Na ₂ SO ₄ added to potassium fluoroacetate	Overnight—about 16 hours	70
8. Fluoroacetic acid neutralized with ammonium hydroxide	About 5 min. after dryness	48

for two hours, a procedure which will completely hydrolyze monochloroacetic acid (3). Fluoroacetic acid was determined in the hydrolysate by the proposed method; 84% was found, compared, with controls of 95–98% (Table 2).

TABLE 2.—*Recovery of monofluoroacetic acid added as the potassium salt to aqueous solution*

ADDED	FOUND
mg	per cent
1.00	98.4
1.00	97.5
1.00	95.5
0+270 mg NaF*	No fluorine found
0.50+270 mg NaF	82

* Quantity of inorganic fluorine in 270 mg NaF is 1000 times the quantity of organic fluorine in 0.5 mg potassium monofluoroacetate.

The somewhat low recovery of fluoroacetic acid (82%) in the presence of a huge excess of inorganic fluoride (Table 2) is believed to be due to incomplete extraction of the fluoroacetic acid from the acidified salts in

the beaker with mobile solvent; at this stage the inorganic fluorine is largely in the form of fluosilicate, which is somewhat insoluble in the 1+1 sulfuric acid.

An experiment was performed to determine the threshold (break-thru) volume, if any, for inorganic fluorides. Fifty mg of inorganic fluorine, one-half in the form of sodium fluoride (55.3 mg) and the other half in the form of potassium fluosilicate (48.3 mg), were placed in a 100 ml beaker, 0.5 ml H_2SO_4 (1+1) added, the material extracted with mobile solvent and chromatographed in the usual way. Fractions of the percolate were tested for fluorine. No trace of fluorine was found in 300 ml of percolate; at this point the collection of fractions was discontinued. Therefore, the chromatographic separation of inorganic fluorine from organic fluorine is complete and there is a wide margin of safety in collecting the fluoroacetic acid fraction.

Measured amounts of fluoroacetic acid as the potassium salt, in the range 0.2–10 p.p.m., were added to various foods and to biological tissues. From foods such as peanuts and cacao beans, which were only washed (not comminuted), recoveries varied from 35 to 70%. Recoveries after comminution of various samples usually fell in the range 90–100%, but in the case of chili peppers, the recovery was markedly lower, about 80% (Table 3). In general, the results indicate that the method is suitable for regulatory problems connected with this compound.

Recovery experiments on flour to which potassium monofluoroacetate was added led to some interesting but not completely explained results. In the early work on flour an enzymatic digestion step was not employed. Recoveries of added fluoroacetic acid in the range 1–10 p.p.m. were 90–100%; however, in all these experiments the analysis was performed within two or three days after the addition of the solution of potassium monofluoroacetate. Subsequently, a collaborative study of the method by several analysts in our laboratories was deemed desirable. Two series of 100 g flour samples were prepared for the study. To each of the samples in one series there was added one ml from a stock solution of potassium monofluoroacetate containing 176 micrograms of fluoroacetic acid per ml and to each sample in the other series 5 ml from the same stock solution, giving a concentration in each series of 1.76 and 8.80 p.p.m. of fluoroacetic acid, respectively. Recoveries obtained by one analyst at the lower level, 1.76 p.p.m., were 97 and 119%, and by a second analyst, 100%. Since the amount of fluorine being determined was rather small, these results are regarded as satisfactory. On the other hand recoveries at the higher level, 8.8 p.p.m., were erratic. Results obtained by one analyst on 9 identical samples were 64, 69, 72, 75, 78, 83, 83, 92, and 95%; by a second analyst on one sample of the same series, 81%; and by a third analyst on one sample of the same series, 72%. However, when the "aged" samples were partially digested with pancreatin before precipitation of proteins with

TABLE 3.—*Recovery of monofluoroacetic acid added as the potassium salt to biological tissue and a wide variety of foods*

PRODUCT	ADDED	RECOVERY
	p.p.m.	per cent
Rat Tissue, skinned and disemboweled carcass ground	10	90
	0	None found
Meat and Bone Scrap	10	94, 98
	5	95
	1	96
	0	None found
Sugar	10	90, 92
	1	88, 89
	0.2	86
Flour	10	93, 97
	1	86, 105
	0.2	94
Wheat	10	95
	1	98
Corn	10	97
	1	94
Cheese	10	92
	1	97
Peanuts	10	93
	1	88
Chili Peppers	10	78
	5	81
Cacao Beans	1	92

phosphotungstic acid, the recoveries were normal: 91, 92, and 92% on the same series at the higher level, 8.80 p.p.m.

An experiment was performed to determine, independently of the method presented here, whether the organic fluorine compound extracted from foods to which potassium monofluoroacetate had been added was actually fluoroacetate. To 100 g samples each of flour and hamburger 20 mg of potassium monofluoroacetate was added. The samples were deproteinized, a 60% aliquot extracted with ether, and the ether extracted with aqueous alkali, in accordance with the method. The aqueous alkaline extract of the ether in the case of hamburger gave a positive indigo test (4); but in the case of flour, a negative test. However, upon repeating the

experiment with flour the quantitative method was followed through the chromatography step, in which instance the alkaline (Na_2CO_3) extract of the percolate gave a positive indigo test. This qualitative test⁵ is highly specific for organic acids containing a reactive alpha halogen atom and indicates that the organic fluorine compound extracted is, in fact, unchanged fluoroacetate.

SUMMARY

A microanalytical method is described for the determination of mono-fluoroacetic acid in foods and biological materials; it is based upon the total fluorine content of an isolated organic acid fraction. Partition chromatography is employed to effect complete separation of fluoroacetate from inorganic acids containing fluorine. The method is highly specific, a quantitative determination being readily made in the presence of a 1000-fold excess of inorganic fluorine. Essentially quantitative results can be obtained on foods and biological materials have a concentration of mono-fluoroacetic acid as low as 0.2 p.p.m.

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PROTECTION OF RIBOFLAVIN FROM DESTRUCTIVE LIGHT RAYS DURING ANALYSIS*

By R. T. OTTES and FLOYD ROBERTS (State Laboratories Department,
Bismarck, North Dakota)

Various investigators have reported the sensitivity of riboflavin to light. All procedures for assay warn that the samples must be protected from light during the course of analysis.

In arranging our laboratory for vitamin analysis we desired to provide proper lighting conditions for riboflavin determinations and still have adequate illumination so that the facilities of the same room could be used for other vitamin determinations which would be in progress simultaneously. We felt that by some means it should be possible to filter out the light rays which are most destructive to riboflavin and yet have ample illumination for other work.

A sample of amber transparent shade, the type commonly used in department store display windows for filtering out ultraviolet rays, was obtained from The Transshade Company, 49 West 27th St., New York, N. Y. The light filtering characteristics of this shade were determined by

⁵ We wish to thank John B. Wilson, Division of Food, for performing this test.

* Presented at the Annual Meeting of The Association of Official Agricultural Chemists, held at Washington, October 10-12, 1949.

checking its spectral absorption. The results are shown in Figure 1. To determine the actual effect of light passing through this shade on riboflavin, preliminary tests were made on a prepared solution containing one p.p.m. of riboflavin in distilled water. One portion of the solution in a small volumetric flask of clear glass was enclosed in a common buff-colored corrugated paper box, having an opening of approximately 3×5 inches cut into its side, and which was covered by the amber shade. The box was placed in a south window so that the entering rays of light would fall directly on the flask through the amber shade. For comparison a

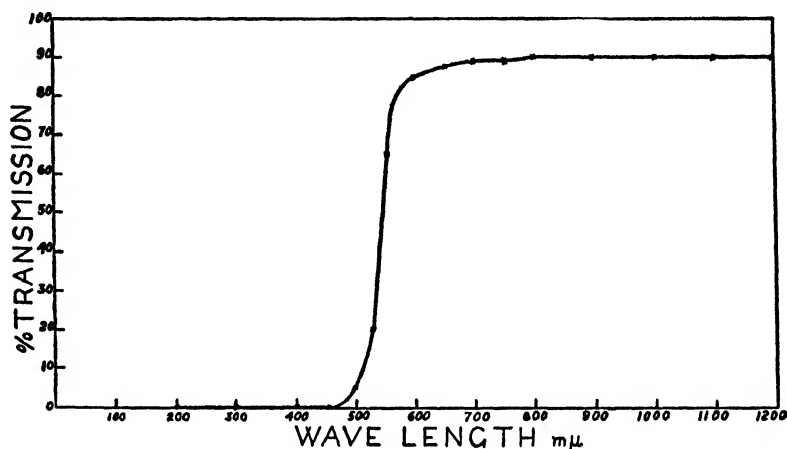


FIG. 1.—Spectral Absorption of Amber Shade

second portion of the solution in a volumetric flask of clear glass and a third portion in a flask of amber glass were also placed in this same window. After exposure to the light of bright sunny days for two hours, three hours, and five days, the amount of riboflavin remaining undestroyed in each flask was determined by placing a portion of the solution from each

TABLE 1.—*Loss of riboflavin in solution placed in direct light of window facing south*

	CONCENTRATION IN P.P.M.				% LOSS		
	INITIAL	2 HRS.	3 HRS.	5 DAYS	2 HRS.	3 HRS.	5 DAYS
Clear Glass	.98	.21	.20	.11	78.6	79.6	88.8
Amber Glass	.96	.75	.67	.17	21.9	30.2	82.3
Amber Shade	1.00	.98	.97	.84	2.0	3.0	16.0

flask directly into fluorometer cells. Fluorescent readings were taken on these cells in a Model DU Beckman spectrophotometer equipped with a fluorescent attachment. These results are given in Table 1.

TABLE 2.—*Loss of riboflavin in solutions placed in diffused light at laboratory bench*

pH		CONCENTRATION IN P.P.M.							PER CENT LOSS						
		INITIAL	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	7 DAYS	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	7 DAYS	
2.2	Control	2.09	2.09	2.09	2.09	2.09	2.09	2.09	0	0	0	0	0	0	
	With Shade	1.97	1.97	1.97	1.97	1.97	1.97	1.97	0	0	0	0	0	0	
	Without Shade	1.97	1.54	.22	.22	.11	.11	.11	21.8	88.8	88.8	94.4	94.4	94.4	
4.0	Control	2.09	2.09	2.09	2.09	2.09	2.09	2.09	0	0	0	0	0	0	
	With Shade	1.97	1.97	1.97	1.97	1.97	1.97	1.97	0	0	0	0	0	0	
	Without Shade	1.87	.55	.38	.33	.33	.33	.33	70.6	79.7	82.5	82.5	82.5	82.5	
6.0	Control	1.98	1.98	1.98	1.98	1.98	1.98	1.98	0	0	0	0	0	0	
	With Shade	1.98	1.98	1.98	1.98	1.98	1.98	1.98	0	0	0	0	0	0	
	Without Shade	1.97	.44	.33	.33	.33	.33	.22	77.7	83.2	83.2	83.2	85.8	88.8	
8.0	Control	2.04	2.04	2.04	2.04	2.04	2.04	2.04	0	0	0	0	0	0	
	With Shade	1.87	1.87	1.87	1.87	1.82	1.82	1.76	0	0	0	2.7	2.7	5.9	
	Without Shade	1.98	.55	.44	.44	.44	.33	.33	72.2	77.8	77.8	77.8	83.3	83.3	

In view of the remarkable possibilities of this shade as shown in the preliminary tests, sheets of the material were procured large enough to cover the window, 40 by 70 inches in size, in the room which was chosen for vitamin assays. A sheet was placed permanently over the upper half of the window. For the lower half the sheet was mounted in a frame so that it could be removed at any time to provide normal light for other work when riboflavin was not involved.

Having adapted the amber shade to the window, additional tests were made with the shade covering the window. The method employed in making the tests was essentially the same as used by Conner and Straub.¹

TABLE 3.—*Effect of added artificial light on riboflavin stability*

pH	% Loss		
	2 HRS.	5 HRS.	7 HRS.
2.2	0	4.6	4.6
4.0	0	6.8	6.8
6.0	0	4.8	4.8
8.0	0	4.8	7.4

Solutions of pure riboflavin (2 p.p.m.) were prepared in phosphate-citric acid buffers ranging in pH values from 2.2 to 8.0. These solutions were kept in clear glass bottles with screw caps. One set of solutions was exposed to diffused light on the work bench, approximately 6 feet from the window, for varying periods of time with the shade covering the window. Another set was used as a control and was kept in a dark cupboard. A third set was tested simultaneously with these, but was placed approximately 6 feet from the window of the same size in an adjacent room, but with no filter over this window. The amount of riboflavin destruction was determined fluorometrically by placing a 1 ml. aliquot of the test solution in 10 ml. of buffer solution of pH 6.0. The results of this work are shown in Table 2.

In order to determine the effect that the use of artificial lights in the laboratory might have on the determination, the above procedure was repeated with the amber shade on the window and with added artificial illumination from two 150-watt ceiling lights under white glass globes. The results of this test are shown in Table 3. Except for possible differences in the intensity and quality of the daylight illumination resulting from the fact that determinations were made on different dates, these results were obtained under the same conditions as were the results in Table 2.

The final tests were actual analyses of an enriched flour and enriched bread. The procedure used was the fluorometric procedure taken from

¹ Conner and Straub, J. Ind. Eng. Chem., Vol. 13, 886, 1941.

Cereal Chemistry, Vol. 22, pp. 455-456. When the amber shade was used, the flour analyzed 2.52 p.p.m. of riboflavin, while with the shade removed, the same sample showed 1.97 p.p.m. of riboflavin, or a 21.8% loss. The bread gave results of 1.83 p.p.m. of riboflavin and 1.06 p.p.m. of riboflavin, respectively, or a 42.1% loss.

SUMMARY

The results of this investigation show the value of a light filter such as the amber transparent sun shade over laboratory windows in minimizing the destruction of riboflavin by light rays during the course of assay according to the method found in *Cereal Chemistry*, Vol. 22, pp. 455-456. Furthermore, such a filter is found to be more effective in preventing destruction of this vitamin than the amber glassware usually provided for this purpose. The quality of the filtered daylight and its intensity is such as to permit normal laboratory work. The use of added artificial light results in appreciable destruction of riboflavin.

A NEW STANDARD FOR VITAMIN D*

By E. M. NELSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The World Health Organization has adopted pure vitamin D₃ as the International Standard for vitamin D in place of the irradiated ergosterol preparation that has been in effect since 1931. For use in this country the U.S.P. Vitamin Advisory Board has recommended the adoption of a similar vitamin D₃ standard that will replace the U.S.P. Reference Cod Liver Oil. This change in the U.S.P. standard will affect the value of the chick unit for vitamin D and it is deemed important to have those concerned with the feeding of poultry, mixing of poultry feeds, and the labeling of vitamin D-containing products for poultry, fully informed of this fact before the new standard becomes effective. It is not possible to give a date when the new standard will become effective, but it may become official before the next A.O.A.C. meeting. The new standard will be announced by the *U. S. Pharmacopoeia* at least six months before it becomes official.

At the First International Conference on Vitamin Standardization, sponsored by the Health Organization of the League of Nations, and held in London in 1931, a special preparation of Irradiated Ergosterol in Oil was adopted as the standard for vitamin D, and the unit for that vitamin was defined in terms of a given weight of that solution. At a Second International Conference on Vitamin Standardization in 1934 the standard previously adopted was retained. It was also decided that in the event that the standard solution needed to be replaced, calciferol or vitamin D₂

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 10-12, 1949.

should be used in making the new standard solution on the basis that .025 microgram of calciferol was equal to 1 unit of vitamin D. However, except for one interval of six months the Irradiated Ergosterol Solution has been issued as the standard.

Since the International Standard was not available in sufficient quantities to meet the needs of the United States, the *U. S. Pharmacopoeia* adopted a specific lot of cod liver oil as a standard for vitamin D. This oil was carefully assayed against the International Standard and 1 U.S.P. unit was defined as being equal to 1 International unit on the basis of rat assays. Subsequently this so-called U.S.P. Reference Cod Liver Oil became the standard for the assay for vitamin D products for poultry because the A.O.A.C. chick assay, which was adopted in 1935, specified its use for determining the potency of such products in A.O.A.C. chick units.

Other lots of U.S.P. Reference Cod Liver Oil were prepared later for use in the same manner as the original U.S.P. Reference Cod Liver Oil. Each lot was assayed against the International Standard for vitamin D by the rat assay and against the previously used Reference Cod Liver Oil by the chick assay before adoption.

It has long been recognized that vitamin D_3 is preferable to D_2 as a standard, because the physiological effect is more uniform in different species of animals. However, vitamin D_3 has not been available in sufficient quantities and in a sufficient degree of purity to serve as a standard until recent years. Two years ago the British Accessory Food Factors Committee initiated an international collaborative study to establish the suitability of vitamin D_3 as an International Standard and to establish its potency in terms of the International Standard. More than 25 grams of pure D_3 obtained from five firms, including two from the United States, was pooled and recrystallized. In the collaborative study, including both chick and rat assays, the potency of this material was compared with that of the International Standard, of pure vitamin D_2 , and of the British Standards Institute's vitamin D_3 standard. In the United States and Canada, under the sponsorship of the Vitamin Advisory Board of the United States Pharmacopoeia, these studies were expanded to include the U.S.P. Reference Cod Liver Oil. Without revealing the identity of this oil, two samples of it were used and instructions were issued for assay of them on the basis that one sample contained 115 and the other 96 units of vitamin D per gram. In all, 54 assays, 29 using rats and 25 using chicks, were conducted by 30 laboratories. Eighteen laboratories in the United States and Canada submitted 13 rat and 16 chick assays. The results of these studies and also chemical and physical studies show that vitamin D_3 is suitable as a standard for vitamin D and that it has a potency very close to 40 million units per gram.

At a meeting of a Committee on Fat-Soluble vitamins, in London in April 1949, it was recommended to the Expert Committee on Biological

Standardization of the World Health Organization that vitamin D₃ be adopted as the International Standard for Vitamin D, and the unit be defined as the vitamin D activity of .025 microgram of vitamin D₃. This report was adopted by the Expert Committee on Biological Standardization. At a meeting of the Vitamin Advisory Board of the United States Pharmacopoeia, in June 1949, it was recommended that vitamin D be adopted as the U.S.P. standard for this vitamin.

If we now use vitamin D₃ as a basis for evaluating the U.S.P. Reference Cod Liver Oil the results of the collaborative studies show that a product assayed against the Reference Oil with rats and found to contain 100 U.S.P. units per gram, will contain 93.4 units when assayed against the new International Standard; and a product found to contain 100 A.O.A.C. chick units per gram when assayed against the present standard will contain 75 units per gram when assayed against the new standard. In other words, four A.O.A.C. chick units are equal to three "International Chick units."

Since supplies of U.S.P. Reference Cod Liver Oil Standard will probably be exhausted in less than a year it became necessary to revise the A.O.A.C. method so as to specify the use of the new U.S.P. Vitamin D₃ Standard. At the same time the name of the unit was changed from the "A.O.A.C. Chick Unit" to the "International Chick Unit."

The adoption of vitamin D₃ as a standard will put the chick unit on a permanent basis because it will be based on a definite weight of a pure and readily reproducible material. It will make for uniformity of labeling of products in international commerce and uniformity in the recording of results in scientific publications in various countries.

NOTE ON CAROTENE PAPER BY DERBY AND DEWITT

By MONROE E. WALL and EDWARD G. KELLEY
(Eastern Regional Research Laboratory,¹ Philadelphia 18, Pennsylvania)

In a recent communication (1), Derby and DeWitt compared a modified Association of Official Agricultural Chemists procedure (2) with a procedure attributed to Wall and Kelley (3) for the extraction of carotene from *dehydrated* alfalfa. The Association of Official Agricultural Chemists method involves extraction by refluxing with 30% acetone in petroleum ether, and the method attributed to Wall and Kelley utilizes cold extraction with ethanol-petroleum ether in the Waring Blendor. The experimental conclusions of Derby and DeWitt are undoubtedly correct, but we feel that their naming of the various extraction procedures is based on an incorrect interpretation of the literature.

In 1941, Moore and Ely (4) presented the method for extraction of carotene with ethanol-petroleum ether in the Waring Blendor. They applied their procedure to both fresh and dehydrated plant material.

In 1943, Wall and Kelley published their carotene procedure (3). Two extraction methods were presented. *Dehydrated* material was extracted by refluxing with 30% acetone in petroleum ether, and fresh tissue was extracted by the Moore and Ely technique (4). They did not apply the Waring Blendor extraction to *dehydrated* products.

It is obvious, therefore, that the modified Association of Official Agricultural Chemists procedure is essentially the same as the Wall and Kelley method for dehydrated products, whereas the procedure attributed to Wall and Kelley is actually the method of Moore and Ely.

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¹ One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

CORRECTIONS IN AUGUST JOURNAL

Page 686. Determination of Gamma-Benzene Hexachloride in Insecticide Products, under "Preparation of Sample (a)," line 2, change "150-200 mg" to read ".75-1.0 g."

BOOK REVIEWS

The Chemistry and Technology of Enzymes. By HENRY TAUBER. John Wiley & Sons, Inc., New York, 1949, 550 pages. Price \$7.50.

This volume, which is an expansion of the author's previous book *Enzyme Technology*, fills an important need, since it not only covers technology of enzymes but also a description of enzymes and their action. A knowledge of enzymes is certainly helpful in their application.

In Part I, "The Chemistry of Enzymes," the chapters are determined by the various changes brought about by enzymes. For example in chapter II, "Esterases," various lipase, liver esterase, tannase, sulfatase, lecithinase, chlorophyllase, various phosphatases, phytase, and cholinesterase are all treated descriptively. References rather than details of the methods of enzyme assay are given.

In Part II, "The Technology of Enzymes," various processes involving enzymes are discussed. This part has no equal in its comprehensive treatment of practical problems involving the use and control of enzyme action. For an example: in the chapter on enzymes in food industries, such things as meat tenderizing, blanching to destroy enzymes in dehydrated and frozen vegetables, enzyme tests for adequacy of blanching, and the role of proteinase and amylase in bread making are considered. Chapters on such relatively recent developments as antibiotic production, and the microbiological assay of vitamins and amino acids, are included, the latter being particularly noteworthy.

Certain errors, mostly of typographical nature, occur throughout the book. Such errors, however, are almost inevitable in such a comprehensive undertaking as this. Little interrelationship between enzymes is given, e.g., the Krebs citric acid cycle is not included. However, in spite of these shortcomings the book is to be recommended particularly to those interested in the use and control of enzyme actions.

EUGENE F. JANSEN

Laboratory Manual Methods of Analysis of Milk and Its Products. 2nd Edition. Published by the Milk Industries Foundation, Washington, D. C., 1949. 627 pages. Price \$15.00.

This is the second edition of the manual, the first of which appeared in 1933. It is a compilation of methods by a committee of members of the Foundation who are actively engaged, or directly concerned, in industry with the control of quality of dairy products. The methods represent best practice in the industry on the chemical and bacteriological methods. The manual, however, includes other procedures to meet the needs of production as well as distribution.

The first part is devoted to an illustrated description of the organization of a milk control laboratory, and a schedule of routine laboratory procedures suggested for control of quality in a market milk distribution plant. This is followed by three parts dealing with bacteriological, chemical, and physical control methods, and a part giving miscellaneous and special tests of dairy products. The greater portion of the text is allotted to these five parts.

The committee drew liberally from the methods of the American Public Health Association and the Association of Official Agricultural Chemists and supplemented these by many procedures from the general literature. No determination the dairy analyst is called upon to make seems to have been overlooked, from the composition, quality and cleanliness of the raw material to the composition, condition, quality and acceptability of the finished dairy products.

To assist the producer and distributor, methods are also included for composition and effectiveness of washing and sterilizing solutions, testing of brine, determin-

ing the strength of milk bottles, and testing of tin plate. The closely allied ice cream industry is provided methods for examining and evaluating gelatin, flavors, sugars, cacao products, and fruits. The sewage disposal problem which, under some conditions, seriously confronts segments of the industry, is recognized by the inclusion of directions for determination of biological oxygen demand.

The book closes with a section on preparation of media, reagents, and standard solutions; numerous conversion and engineering tables; ice cream manufacturing techniques; legal standards; and tables of the properties of dairy and related products.

HENRY A. LEPPER

Biochemical Preparations. Vol. I. HERBERT E. CARTER, Editor-in-Chief. xi+76 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, N. Y., 1949. Price \$2.50.

In the ordinary duties of the regulatory chemist, seldom does the necessity arise for preparing an unusual biochemical compound or reagent which cannot be obtained from a commercial source. When such an occasion does occur, however, it is very helpful to have a source in which one can find a reliable procedure for whatever reagent must be made. Even if this first volume of the series does not contain the specific biochemical preparation in which one is interested at the moment, a similar preparation may be described from which valuable information may be obtained.

The procedure for each preparation in this book has been subjected to limited collaborative study in much the same way as are the official methods of analysis of the A.O.A.C. Thus one can have confidence in the directions. To illustrate the types of synthesis or isolation to be found in this book there may be mentioned: adenosine diphosphate, L-serine and the special reagents used in its isolation from silk fibroin, lycopene, and lysozyme. A total of 17 preparations are described. In addition, references are given for the preparation, as described in *Organic Syntheses*, of 58 other compounds of biochemical interest. The style of presentation resembles in major aspects that of the well-established *Organic Syntheses* (28 volumes); for example, each title and structural formula (where a definite formula can be written) is followed by: I. Principle; II. Starting material; III. Procedure; IV. Properties and Purity of Product; V. Methods of Preparation, and a résumé including references to methods other than the one described in detail.

W. I. PATTERSON

Diagnostic Techniques for Soils and Crops. Edited by Herminie Broedel Kitchen. xxiii+308 pages. American Potash Institute, Washington, D. C. 1948. Price \$2.00.

Diagnostic Techniques for Soils and Crops is a book that should be welcomed by many readers. In addition to its use by soil and plant scientists the book should be useful to County Agricultural agents, Extension workers, Farm Bureau leaders, and many others. Parts of it may, however, be too technical for lay readers.

This volume gives an excellent review of the diagnostic techniques that it is designed to cover. Authors from nine institutions wrote the book. They represent State Institutions, the United States Department of Agriculture, and private industry. Each author is an authority in his field and has done his job splendidly.

The titles of chapters describe the subject matter so well that they are quoted here, some of them with a slightly critical remark, not intended to impart discredit to the high quality of the chapters:

"Chemical Methods for Assessing Soil Fertility" might well include a reference to the original Veitch Method for determination of soil acidity published in 1902, to-

gether with a somewhat more precise statement of the distinctive characteristics of the method.

"Correlation of Soil Tests with Crop Response to Added Fertilizers and with Fertilizer Requirement" tends to emphasize the views of one institution in subject matter and in literature cited to a greater extent than seems desirable in a publication of this kind.

"Operation of a State Soil-Testing Service Laboratory" probably understates the necessity of extensive Statewide knowledge on the part of the operator of the soils of the area and their response to soil-management practices.

"Visual Symptoms of Malnutrition in Plants" would be improved by inclusion of another page or two of colored plates showing additional plants and additional element deficiencies.

Other titles are: "Historical Introduction"; "Operation of an Industrial Service Laboratory for Analyzing Soil and Plant Samples"; "Plant-Tissue Tests as a Tool in Agronomic Research"; "Plant Analysis—Methods and Interpretation of Results"; "Biological Methods of Determining Nutrients in Soils."

A total of more than 500 references are cited. The reviewer believes that this book is destined to be very useful to many persons. He is indeed happy to have a copy for frequent reference.

M. S. ANDERSON

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